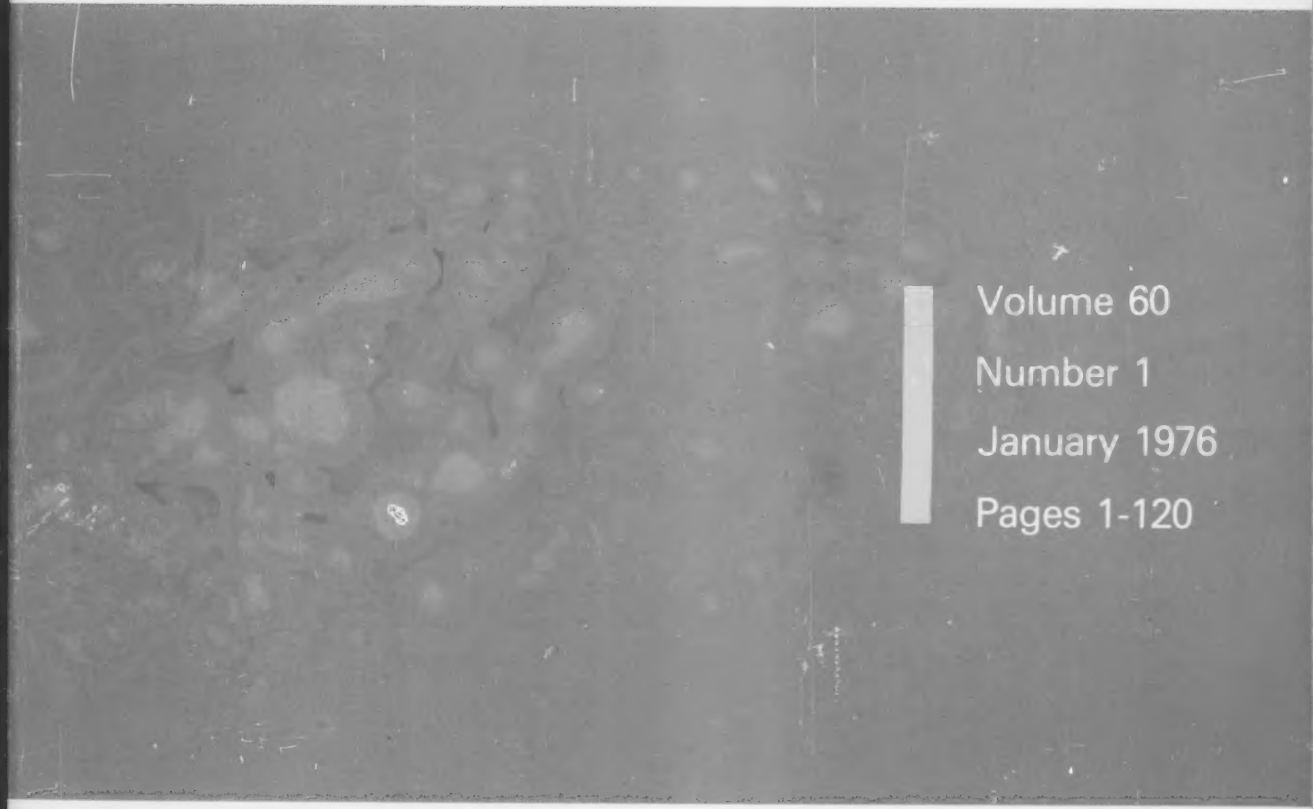
cancer treatment reports



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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

Effects and Specificity of Anticancer Agents on the Respiration and Energy Metabolism of Tumor Cells[1,2]

Mario Gosálvez,* R. García-Cañero, M. Blanco, and C. Gurucharri-Lloyd[3,4]

SUMMARY

Using 35 new anticancer agents from the screening program of the National Cancer Institute we have performed a biochemical study of the effects on the respiration and oxidative phosphorylation of rat liver mitochondria and on the respiration of leukocyte, liver, L1210 leukemia, and Ehrlich ascites cell suspensions. Fifteen of the 35 compounds were found to be potent respiratory inhibitors as defined by 50% inhibition of mitochondrial respiration at concentrations of 110 μ mol/liter or less. The mechanism of respiratory inhibition by the drugs was either a rotenone-, antimycin-, or oligomycin-like effect. One triazine derivative showed some specificity for inhibiting tumor cell respiration in comparison with normal cell respiration. Two naphthoquinone derivatives showed inhibition of respiration in in vivo treatments at chemotherapeutic doses. It was concluded that data on respiratory effects may assist in the interpretation of the results of in vivo and in vitro screening tests of the drugs, and that in some cases, as with the naphthoquinone derivatives, the effects on respiration could be related to the mechanism of action or the mechanism of toxicity of the drugs.

[Cancer Treat Rep 60:1-8, 1976]

In a previous paper (1) we have reported that at low doses the anticancer agents VM-26 and ellipticine affect the respiration and energy metabolism of tumor cells. We suggested that the effect of anticancer agents in respiration might in some cases be the cause for their success in initial screening tests involving mouse ascites tumors. We have also reported that VM-26, already a useful anticancer agent in the clinic, probably has a mechanism of action based on the inhibition of respiration somewhat specific for tumor cells (1-3). We subsequently postulated that anticancer chemotherapy based on specific inhibitors of NADH-linked respiration may be worth investigating (4).

In this report we present a biochemical study characterizing the effect and specificity of several new anticancer agents on the respiration and energy metabolism of tumor cells. The anticancer agents studied were provided by the National Cancer Institute after being selected from those successfully passing initial antitumor screening tests. The purpose of this study was to survey these potentially useful anticancer agents to identify those having significant and specific effects on respiration.

Our results indicate that a substantial percentage of these anticancer agents are highly active or very active respiratory inhibitors. The effects of anticancer drugs on respiration, besides aiding in the interpretation of the results from anticancer screening tests, can in some cases provide information relevant to the mechanism of action, mechanism of toxicity, and biochemical side effects of the compounds.

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[2]Supported by contract N01-CM-53792 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare, and by grant 12-199-75 from the Spanish Instituto Nacional de Previsíon.

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[4]We acknowledge the personal contributions of Mr. J. Barrett (Torrance, Calif) and the discussions of Dr. Vincent H. Bono, Jr (Drug Research and Development Program, DCT, NCI, Bethesda, Md).

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MATERIALS AND METHODS

Measurement of respiration and oxidative phosphorylation in rat liver mitochondria.—Rat liver mitochondria were isolated by the method of Schneider (5). Mitochondrial respiration (1 mg mitochondrial protein/ml) was measured with glutamate plus malate (5 mM) or succinate (5 mM) as substrate in the absence (state 4 respiration) or presence (state 3 respiration) of adenosine-5′-diphosphate (ADP) (0.3 mM). The respiration was measured in a Clark-type oxygen electrode (3-ml chamber) in a medium composed of 225 mM sucrose, 20 mM KCl, 7 mM $MgCl_2$, 10 mM tris-HCl, and 5 mM tris-Pi (pH 7.2, 22°C). The drugs were added in microliter quantities dissolved in 50%/50% dimethylformamide plus dimethylsulfoxide or in water, depending on the drug's solubility. The drug was preincubated with the mitochondria for 8 minutes before the determination of the respiration.

Measurement of Ehrlich ascites tumor cell respiration.—The respiration of Ehrlich ascites tumor cells (20-30 $\times 10^6$ cells/ml, hypertriploid cells) was determined in an oxygen electrode in a medium composed of KCl (6.16 mM), Na_2HPO_4 (9.35 mM), NaH_2PO_4 (1.65 mM), and NaCl (0.9%) (pH 7, 22°C) (6). After 15 minutes of incubation, the respiration rate was compared with the control (untreated cells) respiration rate. All cells used in these experiments were collected from 6-day-old Ehrlich ascites tumors (hypertriploid strain), and hemoglobin-free suspensions were prepared as described by Chance and Hess (6).

Measurement of pig leukocyte respiration.—Mixed populations of leukocytes including lymphocytes were isolated by differential centrifugation from the buffy coat of 2 liters of citrated pig blood. The respiration of the cell suspensions was measured in the Clark-type electrode, using the same medium described for the measurement of Ehrlich ascites tumor cell respiration (6).

Measurement of rat liver cell respiration.—The portal vein and the superior vena cava of rats anesthetized with Nembutal were cannulated in order to perfuse the liver with Krebs-Ringer phosphate salt solution (saturated with oxygen and CO_2). Once the liver was well washed, the perfusion was continued with the same solution supplemented with bovine serum albumin (2.5%), collagenase II (0.05%), and hyaluronidase (0.1%). Afterwards, the liver was isolated from the rat, minced with scissors, and incubated at 37°C for 20 minutes with the Krebs enzyme solution under continuous oxygen gassing. After filtering the cell suspensions with gauze, the isolated liver cells were centrifuged at 1000 rpm and were washed twice with the Krebs-Ringer phosphate medium. The liver cell respiration was measured in a Clark-type oxygen electrode in the same assay medium that was used for the Ehrlich ascites tumor cells.

Measurement of L1210 leukemia cell respiration.—DBA/2 mice bearing L1210 leukemia in ascites form were received from the National Cancer Institute, and the tumors were transplanted to mice (DBA × C3H) by inoculating 10^5 cells ip. After 7 days of tumor growth cells were collected, washed, freed of blood (by a 30-second osmotic shock), and concentrated. Cell respiration was measured in a Clark-type oxygen electrode using the same medium that was employed for the Ehrlich ascites tumor cells.

Measurement of the redox state of mitochondrial cytochrome b.—The redox state of cytochrome b was measured in isolated mitochondria with a double wavelength spectrophotometer at a wavelength pair of 430-410 nm, using the technique described by Chance and Williams (7).

Measurement of the activity of mitochondrial cytochrome c reductase.—The succinate cytochrome c reductase activity present in isolated mitochondria (0.06 mg/ml) was measured by the method of King (8), using oxidized cytochrome c as electron acceptor. The assay medium was composed of phosphate buffer (0.2 M), EDTA (3 mM), cytochrome c (1 mM), and cyanide (1 mM). The absorbancy increase of 550 mμ was measured for at least 3 minutes. Succinate was used to trigger the reaction at concentrations varying from 100 μM to 2 mM. Inhibitors at various concentrations were added to the system prior to the addition of the succinate. The K_i were determined by the Dixon method (9).

Drugs, Solvents, Substrates, and Cofactors

The anticancer drugs used were supplied in concentrated stock solutions in water of *N,N*-dimethylformamide and were added in microliter quantities. Solvents were used as controls. Substrates, salts, and cofactors were purchased from Sigma Chemical Co, St. Louis, Mo. Mitochondrial protein was determined by the biuret reaction (10). Cell concentrations were calculated by cell count in a hemocytometer chamber and after vital staining.

RESULTS

Effects of Anticancer Drugs on Mitochondrial Respiration and Oxidative Phosphorylation

The effect of 35 anticancer drugs on the mitochondrial respiration, in the presence or absence of ADP, with different substrates was determined,

along with their effects on the respiration of Ehrlich ascites cell suspensions.

Table 1 shows the results for the 15 most potent drugs. They include a series of nine 4,6-diaminotriazine derivatives which are potent inhibitors of dihydrofolate reductase (11), two naphthoquinone derivatives (dichloroallyl-lawsone and lapachol), tritylthioalanine, carminomycin, piperazinedione, and methotrexate. The remaining drugs, listed in table 2, required concentrations significantly > 100 μM to inhibit mitochondrial respiration and are considered ineffective in this system.

The structures of the 4,6-diaminotriazine derivatives and their chemotherapeutic activities are shown in table 3. The first five compounds are capable of inhibiting Ehrlich ascites tumor cell respiration by 50% at concentrations ranging from 0.06 to 0.5 mM. All of them inhibit glutamate plus malate or succinate mitochondrial respiration at approximately the same concentration (0.2-0.6 μM). In addition, none of these drugs activate mitochondrial respiration in the absence of ADP (state 4 respiration). They should be considered potent mitochondrial inhibitors, probably acting in the respiratory chain at a site after cytochrome b. The next three drugs are less potent but have the same qualitative properties.

The least potent of the group, NSC-122137, stands out as different. It does not inhibit succinate-dependent respiration and does activate respiration in the absence of ADP, indicating that it both inhibits NADH-linked respiration and uncouples oxidative phosphorylation. This pattern is similar to the effect, previously reported by us (1), of VM-26, an anticancer agent very active in respiration whose mechanism of action very likely depends on its effect on respiration.

Similar to NSC-122137, the drugs tritylthioalanine, carminomycin, and, to a lesser extent, piperazinedione have an action like that of VM-26. The

TABLE 1.—Effect of anticancer drugs on mitochondrial respiration

Drug	Inhibition of mitochondrial respiration with ADP				Activation of mitochondrial respiration without ADP		Inhibition of respiration of Ehrlich ascites cells	
	Glutamate + malate		Succinate					
	μM	%	μM	%	Glutamate + malate	Succinate	mM	%
4,6-Diaminotriazine derivatives								
NSC-127755	0.2	50	0.5	50	no	no	0.068	50
NSC-128571	0.2	50	0.25	50	no	no	0.098	50
NSC-128570	0.25	50	0.25	50	no	no	0.095	50
NSC-113423	0.25	50	0.5	50	no	no	0.078	50
NSC-123461	0.6	50	2	50	no	no	0.470	50
NSC-143010	2	50	2.4	50	no	no	0.0042	50
NSC-139105	7	50	13	50	no	no	0.075	50
NSC-135764	8	50	6	50	no	no	0.300	50
NSC-122137	20	50	—	—	no	yes	—	—
Dichloroallyl-lawsone	2	50	2.5	50	no	yes	0.002	50
Lapachol	7	50	3	50	no	no	0.007	50
Tritylthioalanine	8	50	—	—	no	yes	0.270	50
Carminomycin	25	50	—	—	yes	yes	—	—
Piperazinedione	86	50	300	50	no	yes	0.220	50
Methotrexate	110	50	150	20	no	no	1.4	50

TABLE 2.—Drugs ineffective in respiration

Adriamycin-DNA*	NSC-104967
Daunorubicin-DNA*	Anguidine
Thalicarpine	Inosine dialdehyde
Asaley	Bleomycin
Tetrandrine	Dianhydrogalactitol
Cytembena	2-Amino-1,3,4-thiadiazole
Chromomycin A_3	Rubidazone
Sangivamycin	NSC-79423
Gallium nitrate	Cyclocytidine
Hexamethylmelamine	Neocarzinostatin

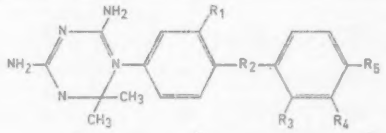
*Concentrations for assay were based on adriamycin or daunorubicin content.

two naphthoquinone derivatives dichloroallyl-lawsone and lapachol have a pattern like that of the triazines and probably act after cytochrome b in the respiratory chain. Methotrexate, a much less potent inhibitor than the 2,4-diaminotriazines, is also an inhibitor of NADH-linked respiration (table 1), as has been previously reported by Vogel et al (12). However, it does not uncouple oxidative phosphorylation.

Further mechanistic studies were done in mitochondria in order to pinpoint the site of action in the mitochondrial respiratory chain of lapachol. It was found that lapachol does not release the respiratory inhibition induced by oligomycin. In addition, the uncoupler dinitrophenol released the inhibition of respiration caused by lapachol. This pattern of effects implies that lapachol has an oligomycin-like action in mitochondria. Similar experiments were done with the lapachol derivative dichloroallyl-lawsone. The inhibitory effect of this compound was also released by dinitrophenol. However, this compound was also capable of releasing the oligomycin inhibition. The effects of dichloroallyl-lawsone corroborate the results obtained by Howland (13) using hydroxyalkylnaphthoquinones. The effect of lapachol and its derivative on the redox state of mitochondrial cytochrome b, in the presence or absence of ADP, was determined. Both agents convert cytochrome b to its reduced form in the presence of ADP but not in its absence, supporting the proposed oligomycin-like effect of these compounds.

Figure 1 shows the effects of the triazine derivatives on mitochondrial cytochrome c reductase as recorded in the spectrophotometer, following the disappearance of cytochrome c. The ordinate corresponds to optical density changes and the abscissa corresponds to time. The control recording of the enzyme's activity is depicted at the far left of the figure. The rest of the recordings indicate the inhibition of the enzyme's activity with low concentrations of various antifolate drugs. The K_i of these drugs for mitochrondrial cytochrome c reductase were estimated to range between 2×10^{-6} M (NSC-123461) and 2×10^{-4} M (NSC-139105). The effect of the drugs on the enzyme was found to be time dependent; pre-incubating the drugs with the enzyme made it possible to obtain K_i for the drugs which were 1-3 orders of magnitude lower than the K_i obtained without incubation. It is significant that, just as methotrexate is also an inhibitor of NADH-linked mitochondrial respiration, these new tria-

TABLE 3.—Activity of triazine derivatives in mouse tumors



Triazine derivatives	R_1	R_2	R_3	R_4	R_5	Activity* in— L1210 leukemia	P388 leukemia	B16 melanoma
NSC-139105	Cl	-O-CH_2-	H	-CO-NH$(CH_2)_2$	H	I	I	I
NSC-143010	Cl	-O-CH_2-	H	-CO-NH$(CH_2CH_3)_2$	H	I	I	—
NSC-113423	H	$(CH_2)_2$-CO-NH-	H	CH_3	-SO_2F	I	I	I
NSC-127755	Cl	-$(CH_2)_4$-	Cl	H	-SO_2F	A	A	A
NSC-128571	Cl	-$(CH_2)_4$-	H	-SO_2F	Cl	I	A	A
NSC-128570	Cl	-$(CH_2)_4$-	H	-SO_2F	H	I	A	I
NSC-123461	Cl	-$(CH_2)_2$-CO-NH-	H	H	-SO_2F	I	A	I
NSC-135764	Cl	-O-CH_2--CO-NH-	H	-SO_2F	H	A	A	A
NSC-122137	H	-CH_2-NH-CO-NH-	H	-SO_2F	H	A	A	A

*A = active; I = inactive.

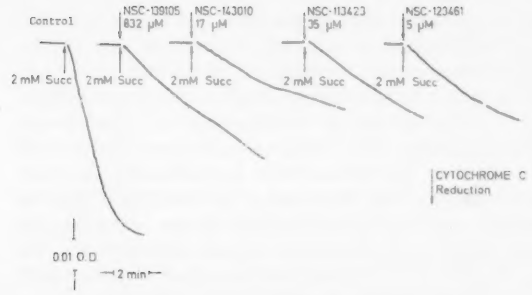


FIGURE 1.—Effect of various 4,6-diaminotriazine derivatives on the reduction of cytochrome c by succinate in the presence of mitochondrial cytochrome c reductase (0.06 mg mitochondrial protein/ml). The reaction (disappearance of cytochrome c recorded by the decrease of absorbancy at 550 mμ) was triggered with succinate. The ordinate represents a diminution of optical density and the abscissa represents minutes.

zine derivatives, developed by Baker (11) as inhibitors of dihydrofolic acid reductase, are also active in a mitochondrial reductase.

Effect and Specificity of Anticancer Drugs on Tumor Cell Respiration

The studies in mitochondria permit a precise formulation of the level of activity and mechanism of action of the different drugs in mitochondrial respiration. However, to ascertain the biologic significance of the mitochondrial effects of the drugs, it is pertinent to study the compounds in in vitro and in vivo whole cell respiration. A very active drug in mitochondria could not have a biologically significant effect on respiration if it were unable to enter in the cell or were inactivated by cellular metabolism before reaching the cell's mitochondria. The effects of these drugs on the respiration of suspensions of living pig leukocytes, rat hepatocytes, Ehrlich ascites tumor cells, and L1210 leukemia cells were studied. To allow for the comparison of the effect of each drug on the four different cell preparations, the concentration of the cells in each case was adjusted to yield equivalent respiration (1). The respiratory measurement was determined after the cells had been respiring for 15 minutes in the presence of the drug.

Table 4 includes data representing the specificity of each drug for each cell preparation relative to the other cells. In each case, the specificity is defined by two numbers: the relative specificity factor (RSF) and the absolute specificity factor (ASF). The RSF is calculated by the ratio between the concentration necessary to inhibit 80% normal cell respiration and the concentration necessary to inhibit 80% tumor respiration. The ASF is calculated by the ratio between the maximum concentration with no effect on respiration of normal cells and the minimum concentration required for 80%

TABLE 4.—Effects of anticancer drugs on respiration of various cell suspensions

	Leukocytes/ Ehrlich ascites cells		Leukocytes/ L1210 cells		Liver cells/ Ehrlich ascites cells		Liver cells/ L1210 cells		Liver cells/ leukocytes		L1210 cells/ Ehrlich ascites cells	
Drug	RSF	ASF	RSF	ASF	RSF	ASF	RSF	ASF	RSF	ASF	RSF	ASF
NSC-127755	2	0.1	5.7	0.5	2	0.06	7.2	0.2	1.2	0.035	0.28	0.03
NSC-128571	1	0.035	2	0.07	0.9	0.12	3.5	0.4	1.9	0.23	0.28	0.02
NSC-128570	1.1	0.045	5.6	0.2	2	0.34	5.7	1	1.8	0.32	0.34	0.034
NSC-113423	8	0.1	10	0.6	1.4	0.4	1	0.4	0.1	0.04	1	0.06
NSC-123461	0.6	0.12	4.5	1	3	0.9	34	2.5	7.1	1.3	0.1	0.3
NSC-143010	6	0.5										
Dichloroallyl-lawsone	1	0.008										
NSC-139105	13.3	0.075										
Lapachol	3	0.5										
NSC-135764	0.3	0.06										
Tritylthio-alanine	2.1	0.66										

inhibition of tumor cell respiration. The table also shows the RSF and ASF calculated between two kinds of normal cells and two kinds of tumor cells. Some of the concentrations required for 80% inhibition of normal cells were extrapolated from the shape of the inhibition curve of the drugs. RSF ratios of 10 or greater indicate a tenfold difference in sensitivity of the normal and tumor cells. Three of the triazine derivatives, NSC-113423, NSC-123461, and NSC-139105, have this characteristic. An ASF ratio of 1 or greater indicates no effect on normal cells at a concentration having very significant effects on tumor cells. Of the drugs tested, only the triazine NSC-123461 was in this class.

In Vivo Effects

To determine if the in vitro effects of an anticancer agent in respiration are of biologic significance, it is desirable to assay the effect of the drug on the respiration of tumor cells isolated from the animals treated in vivo with the drug at chemotherapeutic doses. Figure 2 shows these experiments performed with various anticancer drugs. The left side of the figure indicates that cells isolated from Ehrlich ascites tumors 2 hours after the animal has been treated with the drug show normal cell respiration when the animal is treated with the antifolates NSC-139105, NSC-143010, and NSC-113423 at chemotherapeutic doses. However, treatment with the naphthoquinone compounds lapachol and dichloroallyl-lawsone induces a progressive inhibition of respiration with increasing doses. The right side of the figure shows the time course of the effect in in vivo respiration of ip injections of 15 mg/kg of each of the four drugs. It can be seen that all drugs cause an intense respiratory inhibition during the first half hour and that this inhibition is released with time, except for lapachol, whose inhibition remained constant. These results are interpreted as an inactivation, an excretion, or a metabolism of the drugs. Alternatively, the drug could be equilibrated throughout the rest of the mouse's body so that the concentration became so low that there was no longer respiratory inhibition except for lapachol and dichloroallyl-lawsone. These agents were the most potent on Ehrlich ascites cells in vitro, which may reflect a preferential uptake in the cells.

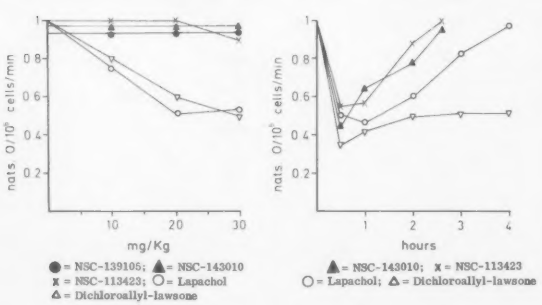


FIGURE 2.—*Left*: Effect of increasing ip doses of the drugs on the respiration of Ehrlich ascites tumor cells isolated from the tumor 2 hrs after being treated with the drugs. *Right*: Effect of ip doses of 15 mg/kg of the drugs on the respiration of Ehrlich ascites tumor cells isolated from the treated tumors at various intervals after the injection of the drugs.

DISCUSSION

Our studies have shown that a substantial number (14 of 35) of the anticancer agents studied are highly active or very active respiratory inhibitors with different sites of action in the respiratory chain and energy transport pathway. The nine triazine derivatives listed in table 1 block the respiratory chain after cytochrome b, probably by inhibiting cytochrome c reductase. Lapachol and dichloroallyl-lawsone, two naphthoquinone derivatives, behave as oligomycin-type inhibitors. Tritylthioalanine, carminomycin, and piperazinedione behave as inhibitors of NADH-linked respiration because they either had no effect on succinate respiration or only inhibited it at very high concentrations. The effect of these agents on respiration may, by itself, explain their passing initial screenings involving tissue culture or ip injections of the drug into mouse ascites tumors, since it is well known that under these conditions, respiratory inhibitors are able to induce the arrest of cell growth (4,14). Thus, data on respiratory effects may assist in the interpretation of the results of in vitro and in vivo screening tests carried out with the drugs. Additionally, the effect on respiration may be relevant in some cases for some of the toxic or secondary effects of the drugs.

It would appear that the inhibition of respiration in in vivo treatments with chemotherapeutic doses of a drug, such as that described for lapachol and its derivative, would be strong evidence supporting a mechanism of action of the drug related to its effects on respiration. However, the abdominal cavity of mice with ascites tumors might behave in a fashion similar to that noted in an in vitro chamber if the drug is not rapidly absorbed. In order to ascertain convincingly the biologic significance of the effect of the drug in respiration, it would be desirable to demonstrate in vivo and in situ that it inhibits the respiration of parenterally treated solid tumors, as we have done with VM-26 (3). However, until these experiments

are done, lapachol should be considered a drug with a possible mechanism of action or mechanism of toxicity related to its effects on respiration. The triazine compounds inhibit the enzyme dihydrofolate reductase in the nanomolar range (11), thus, their effect on respiration must be considered as a secondary biochemical effect occurring at relatively high concentrations. However, with these compounds, some specificity has been found for tumor cell respiration in comparison with normal cell respiration. The occurrence of compounds with such specificity encourages the investigation of anticancer chemotherapy based on respiratory inhibitors (4). The specificity was found by comparing normal and tumor cells of different species. Although the respiration of cells and mitochondria from different species is highly comparable, as long as equivalent respiration is assured (1), a definitive assessment of specificity should include the comparison of tumor and normal tissues from the same animal, as has been done for VM-26 (3).

LIST OF COMPOUNDS

Adriamycin-DNA: NSC-169534; nucleic acid, deoxyribo-, sodium salt, compd. with (8*S-cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride

2-Amino-1,3,4-thiadiazole: NSC-4728; CAS reg. No. 4005-51-0

Anguidin: NSC-141537; CAS reg. No. 22135-66-6; spiro[2,5-methano-1-benzoxepin-10,2′-oxirane]-5α-(6*H*)-methanol, 2,3,4,5,7,9α-hexahydro-3,4-dihydroxy-5,8-dimethyl-, 4,5α-diacetate

Asaley: NSC-167780; CAS reg. No. 3577-89-7

Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

Carminomycin: NSC-180024; 5,12-naphthacenedione, 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranoxyl)oxy]-7,8,9,10-tetrahydro-1,6,8,11-tetrahydroxy-, (8*S*-cis)-

Chromomycin A_3: NSC-58514; CAS reg. No. 7059-24-7

Cyclocytidine: NSC-145668; CAS reg. No. 10212-25-6; 6*H*-furo[2′,3′:4,5]oxazolo-[3,2-*a*]pyrimidine-2-methanol, 2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-, monohydrochloride

Cytembena: NSC-104801; CAS reg. No. 16170-75-5; acrylic acid, 3-*p*-anisoyl-3-bromo-, sodium salt

Daunorubicin-DNA: NSC-169533; nucleic acid, deoxyribo-, sodium salt, compd. with (8*S*-cis)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride

Dianhydrogalactitol: NSC-132313; CAS reg. No. 23261-20-3; galactitol, 1,2:5,6-dianhydro-

Dichloroallyl-lawsone: NSC-126771; CAS reg. No. 36417-16-0; 1,4-naphthoquinone, 2-(3,3-dichloroallyl)-3-hydroxy-

Gallium nitrate: NSC-15200; CAS reg. No. 13494-90-1

Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6

Inosine dialdehyde: NSC-118994; diglycoaldehyde, α-(hydroxymethyl)-$_D$-(6-hydroxy-9*H*-purin-9-yl)-

Lapachol: NSC-11905; CAS reg. No. 84-79-7; 1,4-naphthoquinone, 2-hydroxy-3-(3-methyl-2-butenyl)-

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Neocarzinostatin: NSC-157365; CAS reg. No. 4507-13-5

NSC-79423: CAS reg. No. 6994-00-9; ethanol, 2,2′-[(*p*-nitrosophenyl)imino]di-, dimethanesulfonate

NSC-104967: CAS reg. No. 22964-45-0; acetanilide, 3′-[bis(2-hydroxyethyl)amino]-, dimethanesulfonate (ester)

NSC-113423: CAS reg. No. 21321-03-9; ethanesulfonic acid, compd. with 4-[*p*-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)hydrocinnamamido]-*o*-toluenesulfonyl fluoride (1:D; Baker's antifol

NSC-122137: CAS reg. No. 21316-21-2; ethanesulfonic acid, compd. with *N*-[[*p*-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)benzyl]-carbamoyl]metanilyl fluoride (1:1)

NSC-123461: CAS reg. No. 21320-47-8; ethanesulfonic acid, compd. with *N*-[2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)hydrocinnamoyl]sulfanilyl fluoride (1:1)

NSC-127755: CAS reg. No. 31368-48-6; ethanesulfonic acid, compd. with 3-chloro-4-[4-[2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)phenyl]butyl]benzenesulfonyl fluoride (1:1)

NSC-128570: CAS reg. No. 31368-50-0; ethanesulfonic acid, compd. with *m*-[4-[2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)phenyl]butyl]benzenesulfonyl fluoride (1:1)

NSC-128571: CAS reg. No. 31368-51-1; ethanesulfonic acid, compd. with 2-chloro-5-[4-(2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)phenyl]butyl]benezenesulfonyl fluoride (1:1)

NSC-135764: CAS reg. No. 30885-66-6; ethanesulfonic acid, compd. with *N*-[α-[2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)phenoxy]-*p*-toluoyl]metanilyl fluoride (1:1)

NSC-139105: CAS reg. No. 41191-04-2; ethanesulfonic acid, compd. with α-[2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)phenoxy]-*N,N*-dimethyl-*m*-toluamide (1:1)

NSC-143010: CAS reg. No. 41191-05-3; ethanesulfonic acid, compd. with α-[2-chloro-4-(4,6-diamino-2,2-dimethyl-*s*-triazin-1(2*H*)-yl)phenoxy]-*N,N*-diethyl-*m*-toluamide (1:1)

Piperazinedione: NSC-135758; CAS reg. No. 41109-80-2; 2,5-piperazinedione, 3,6-bis-(5-chloro-2-piperidyl)-, dihydrochloride

Rubidazone: NSC-164011; CAS reg. No. 36508-71-1; daunorubicin, benzoylhydrazone, monohydrochloride

Sangivamycin: NSC-65346; CAS reg. No. 17222-10-5

Tetrandrine: NSC-77037

Thalicarpine: NSC-68075; CAS reg. No. 5373-42-2

Tritylthioalanine: NSC-83265; CAS reg. No. 2799-07-7; L-alanine, 3-(tritylthio)-

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Detection of Bacterial Endotoxin in Antitumor Agents[1,2,3,4]

Stuart E. Siegel,* Ronald Nachum, Suzanne Leimbrock, and Myron Karon[5]

SUMMARY

The *Limulus amebocyte* lysate assay was used to detect the presence of bacterial endotoxin in a variety of chemotherapeutic agents currently in use. Results were quantitated using a standard *Escherichia coli* endotoxin curve. Relative concentrations of endotoxin ranging from 0.1 to 63 ng/ml were detected in individual lots of L-asparaginase, 5-azacytidine, bleomycin, DTIC, actinomycin D, adriamycin, and vinblastine diluted to normal concentrations for injection. When multiple lots of a given drug were tested, at least one lot produced a negative assay, indicating <0.1 ng/ml of endotoxin in 50% of these drugs. All lots of cytosine arabinoside, cyclophosphamide, daunorubicin, vincristine, and streptozotocin tested had < 0.1 ng/ml of endotoxin. Inactivation kinetics of the *Limulus*-positive drug material in dilute alkali solutions were identical to those of *E. coli* endotoxin. The *Limulus* lysate test is a rapid and specific method for the detection of small amounts of bacterial endotoxin contaminating parenteral preparations of antitumor agents.

[Cancer Treat Rep 60:9-15, 1976]

Adverse reactions to chemotherapeutic agents now in use are often poorly understood. Fever, chills, and hypotension have frequently been observed following the parenteral administration of certain agents, primarily L-asparaginase and bleomycin (1-7). Since many of these drugs are the products of microbial fermentation, contamination with bacterial pyrogen or endotoxin has been suspected (1). Standard pyrogen testing using the rabbit bioassay system has failed to detect small quantities of endotoxin in most cases; this method can only reliably detect concentrations of 0.01-0.1 μg/ml or greater (8-11).

In contrast, the *Limulus amebocyte* lysate assay described by Levin et al specifically detects endotoxin elaborated by gram-negative bacteria at concentrations as low as 0.1 ng/ml (12-15). In this study the *Limulus* assay was used as a tool to determine the frequency and quantity of endotoxin contamination in a number of commonly used chemotherapeutic agents.

MATERIALS AND METHODS

Limulus Lysate Assay

Using the procedure of Levin et al with minor modifications, *Limulus* assays were performed on parenteral drug solutions by mixing 0.03 ml each of *Limulus* lysate (Woods Hole Marine Biological Laboratories, Woods Hole, Mass, lot No. 27-5) and the parenteral drug preparations dissolved in pyrogen-free 0.011 M potassium phosphate buffer (pH 7.4, McGaw), incubating at 37°C for 4 hours and then at 20°C for 20 hours (12-16). The assays were interpreted as follows: negative = clear or slight flocculation; 3+ = solid gel; 2+ = adherent "starchy" granules; and 1+ = increased turbidity and viscosity.

Pyrogen-free glassware and disposable plastic materials were used at all times. The endotoxin concentration of each drug solution was determined by the method of Reinhold and Fine (17). Assays of serial twofold drug dilutions were compared with a standard *E. coli* endotoxin curve (*E. coli* 0127:B8, Difco) (fig 1). The highest dilution

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[4]Drugs used in this study were supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI.

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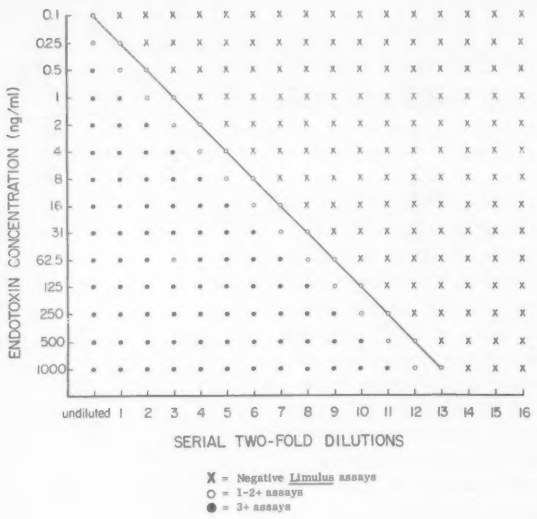

FIGURE 1.—Standard curve for determination of endotoxin concentration, using serial twofold dilutions of *E. coli* endotoxin. Concentrations were obtained by determining the highest dilution yielding a 1-2+ reaction (straight line).

producing a 1+ reaction after a 24-hour incubation period was interpreted as the end point at each endotoxin concentration. All determinations were performed twice. Under these conditions, as little as 0.1 ng/ml of *E. coli* endotoxin was reliably detected. A negative control assay consisting of potassium phosphate buffer and lysate alone was included with each assay system.

Antitumor Agents

All drugs were dissolved in 0.011 M pyrogen-free potassium phosphate buffer (pH 7.4) within 1 hour prior to testing. Final concentrations of individual agents were similar to those recommended for clinical use (table 1). At least two separate vials of each drug lot were tested and each assay was performed twice.

RESULTS

Drug Assays

Positive *Limulus* assays were obtained with a wide range of unrelated chemotherapeutic agents (table 1). Concentrations ranging from 0.1 to 63 ng/ml were observed in individual lots of L-asparaginase, 5-azacytidine, bleomycin, vinblastine, actinomycin D, DTIC, and adriamycin. The amounts of endotoxin detected were not affected by prior extraction of the drug with chloroform. Of particular interest was the wide range of endotoxin concentrations observed in different lots of these drugs. Fifty percent of the drugs in which multiple lots were available for testing had at least one lot which yielded a negative *Limulus* assay, indicating <0.1 ng/ml of endotoxin. The highest endotoxin concentrations were found in individual lots of vinblastine, bleomycin, and L-asparaginase.

Negative assays were obtained with all lots of cytosine arabinoside, cyclophosphamide, daunorubicin, vincristine, and streptozotocin, although only a single lot was available for testing for all but one of these agents. Of particular interest, vinblastine lot No. 4WT55A was reported to be nonpyrogenic when injected iv at a dose of 0.3 mg/kg in three rabbits.[6]

Inactivation Kinetics

Since previous studies have suggested that certain polynucleotides and thromboactive substances may also produce positive *Limulus* tests (18), we attempted to further establish the identity of the "*Limulus*-positive material" in the various drug preparations as endotoxin by comparing the pattern of loss of *E. coli* endotoxin gelation activity caused by various physical conditions with that of the drug solutions.

Each sample of endotoxin or drug was incubated in sterile, pyrogen-free 0.011 M potassium phosphate buffer (pH 7.4, McGaw), sterile, pyrogen-free 0.1 N NaOH (Sigma) at 100°C, and 0.1 N NaOH at 20°C in sealed pyrogen-free glass ampules. Aliquots were removed at various time intervals, buffered to pH 7.0-7.2 with sterile, pyrogen-free 0.1 N HCl (Sigma), and assayed for endotoxin concentration with *Limulus* lysate as previously described. The log of the endotoxin concentration in ng/ml was plotted against time in minutes (fig 2). No loss of activity was noted when endotoxin was incubated at 20°C for 1 hour in 0.1 N NaOH, while a rapid, complete loss of activity occurred with boiling in the same solution. A slow 1-log loss of activity was noted with boiling in potassium phosphate buffer for 1 hour.

Limulus-positive lots of L-asparaginase, bleomycin, vinblastine, actinomycin D, and 5-azacytidine were identically treated and produced remarkably similar results to those of the *E. coli* endotoxin. Representative curves are shown in figure 3. Boiling at 100°C with N NaOH produced a rapid fall in endotoxin concentration within 1 hour with all positive drug lots tested, while the same treatment of the drug in neutral buffer resulted in a slow 1-log or less activity loss.

[6]Dyke RW. Personal communication.

TABLE 1.—Endotoxin concentration in chemotherapeutic agents*

	Lot No.	Drug concentration/ml	Endotoxin concentration (ng/ml in *E. coli* endotoxin equivalent)	Usual dosage in man/m²
Bleomycin	72L528	15 units	5.0	15 units
	72L216	15 units	5.0	
	72L481	15 units	2.0	
	73L510	15 units	<0.1	
	G3689	15 units	<0.1	
	73L511	15 units	<0.1	
L-Asparaginase	C-0390	5000 units	4	6000-60,000 units
	C-B311	5000 units	0.5	
	C-A973	2000 units	(+)	
Vinblastine	4WT55A	1 mg	63	2 mg
	8JR31B	1 mg	0.5	
	6TE15A	1 mg	<0.1	
	80C01A	1 mg	<0.1	
Adriamycin	38	1 mg	0.5	30-90 mg
	42	1 mg	(+)	
Daunorubicin	156	2 mg	<0.1	30-90 mg
	155	2 mg	<0.1	
	158	2 mg	<0.1	
Actinomycin D	0099P	0.5 mg	0.25	500 μg
	0761P	0.5 mg	2.0	
	0521R	0.5 mg	2.0	
DTIC	BV-72-267	10 mg	0.25	250 mg
Cytosine arabinoside	BV-68-230	20 mg	<0.1	100-150 mg
5-Azacytidine	BV-73-234	10 mg	0.5	100 mg
	BV-72-242	10 mg	1.0	
	BV-72-208	5 mg	(+)	
	BV-72-205	10 mg	(+)	
	BV-73-218	10 mg	(+)	
	BV-73-220	10 mg	<0.1	
Cyclophosphamide	MDE49B	20 mg	<0.1	100-1200 mg
Vincristine	6PD95A	1 mg	<0.1	2 mg
Streptozotocin	BV-71-221	100 mg	<0.1	1-4 g

*Relative endotoxin concentrations were determined by *Limulus* assay of serial twofold drug dilutions, using the standard *E. coli* endotoxin curve (*E. coli* 0127:B8, Difco). (+) = a positive assay on an undiluted drug sample was obtained, but further quantitation was not performed.

DISCUSSION

This study has shown the sensitivity and specificity of the *Limulus* lysate assay in detecting and quantitating endotoxin contamination of chemotherapeutic drug preparations. This specificity was recently questioned by Elin and Wolff who showed that compounds such as poly IC, thrombin, and ribonuclease, in addition to endotoxin, can produce positive *Limulus* assays (18). However, these agents lost their ability to produce gelation following chloroform extraction, a process which does not remove bacterial lipopolysaccharides. In the present study, chloroform extraction uniformly

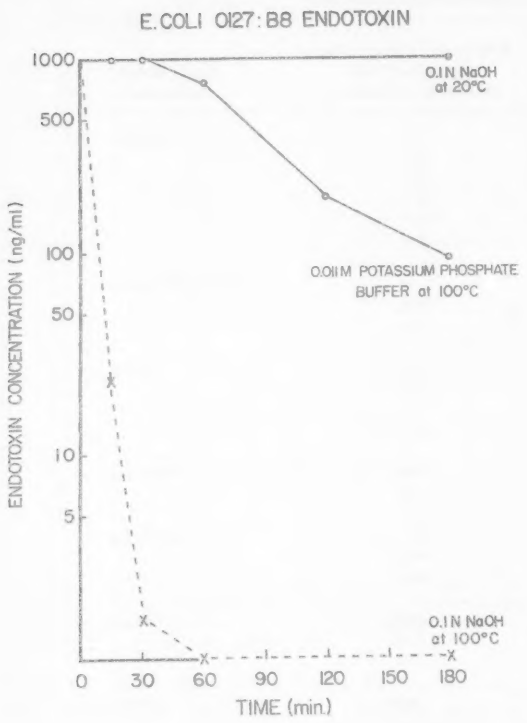

FIGURE 2.—Inactivation of kinetics of *E. coli* endotoxin (*E. coli* 0127:B8, Difco). Endotoxin concentrations were determined by *Limulus* assay of serial twofold drug dilutions using the standard *E. coli* endotoxin curve.

failed to alter quantitative kinetic results. Furthermore, the marked diversity of chemical structure, mechanism of action, and source of the positive drugs in this study renders the possibility of nonspecific gelation unlikely.

Bacterial endotoxin has a unique pattern of detoxification that has been used to partially separate the various components of the lipopolysaccharide molecule. Treatment with weak alkali or acid solutions at 20°C produces an immediate drop in particle size, but no loss of pyrogenicity is noted until after 3 hours of treatment is given (19-26). The subsequent loss of pyrogenicity, complete after 8 hours of weak alkali hydrolysis, is correlated with the degradation of the lipid-A component of the endotoxin molecule (20,22,24-28). It is this biologically active lipid-A moiety that is also responsible for endotoxin-mediated gelation of the *Limulus* lysate (29). Similarly, boiling in neutral buffer produces no loss of pyrogenicity, while a rapid drop in activity occurs when endotoxin is boiled in dilute alkali or acid solutions (19-28). In this study, *Limulus* assay-positive drugs showed virtually the same unique pattern of endotoxin degradation as did purified *E. coli* endotoxin under these same conditions (specifically, losing activity rapidly and completely when boiled in dilute alkali).

Dinarello et al recently suggested that bleomycin-induced fever is due to the release of an endogenous leukocyte pyrogen (30). In their study, pyrogenic tolerance to bleomycin in rabbits could not be shown and the *Limulus* assay was reported as negative although detailed data on this point were lacking. In the present study, *Limulus* assays of the *same* drug lot (No. 71-L-481) used by these authors were consistently positive, although the concentration of endotoxin present was only 2 ng/15 units of drug (table 1). Despite these minute concentrations, these seemingly contradictory *Limulus* lysate results could be attributed to differences in the sensitivity of the lysate reagents used, a phenomenon well documented in the past (31,32).

With the exception of a single vinblastine lot, it is not surprising that previous pyrogen testing with rabbits has not detected endotoxin contamination in the agents assayed in the present study, since the concentrations of endotoxin present are almost all below the demonstrated sensitivity of the bioassay system (8-10). Nor is it unreasonable that some variability in endotoxin concentrations from lot to lot of a given drug was noted. Methods of drug synthesis, whether microbial or chemical, are often performed using glassware or media that are sterile, but *not* nonpyrogenic. In particular, column chromatographic materials often become heavily contaminated with repeated use. Most pharmaceutic laboratories presently employ methods of heat and acid treatment to render their synthetic systems nonpyrogenic, but they are often limited by lack of stability of the chemotherapeutic agents under these conditions. We have recently shown that *Limulus* lysate itself has the ability to remove endotoxin from solution, and it may prove to be useful in the further decontamination of pharmaceutic agents (33).

The level of *E. coli* endotoxin equivalents detected in vinblastine lot No. 4WT55A would be expected to yield a positive rabbit pyrogen test, since it is 19 times higher than the pyrogenic threshold dose for *E. coli* endotoxin (11). This discrepancy in the results of the two assays remains unexplained at this time. As previously noted, it is unlikely that the *Limulus* gelation activity is due to substances other than endotoxin. The limited amount of this vinblastine lot available prevented indepen-

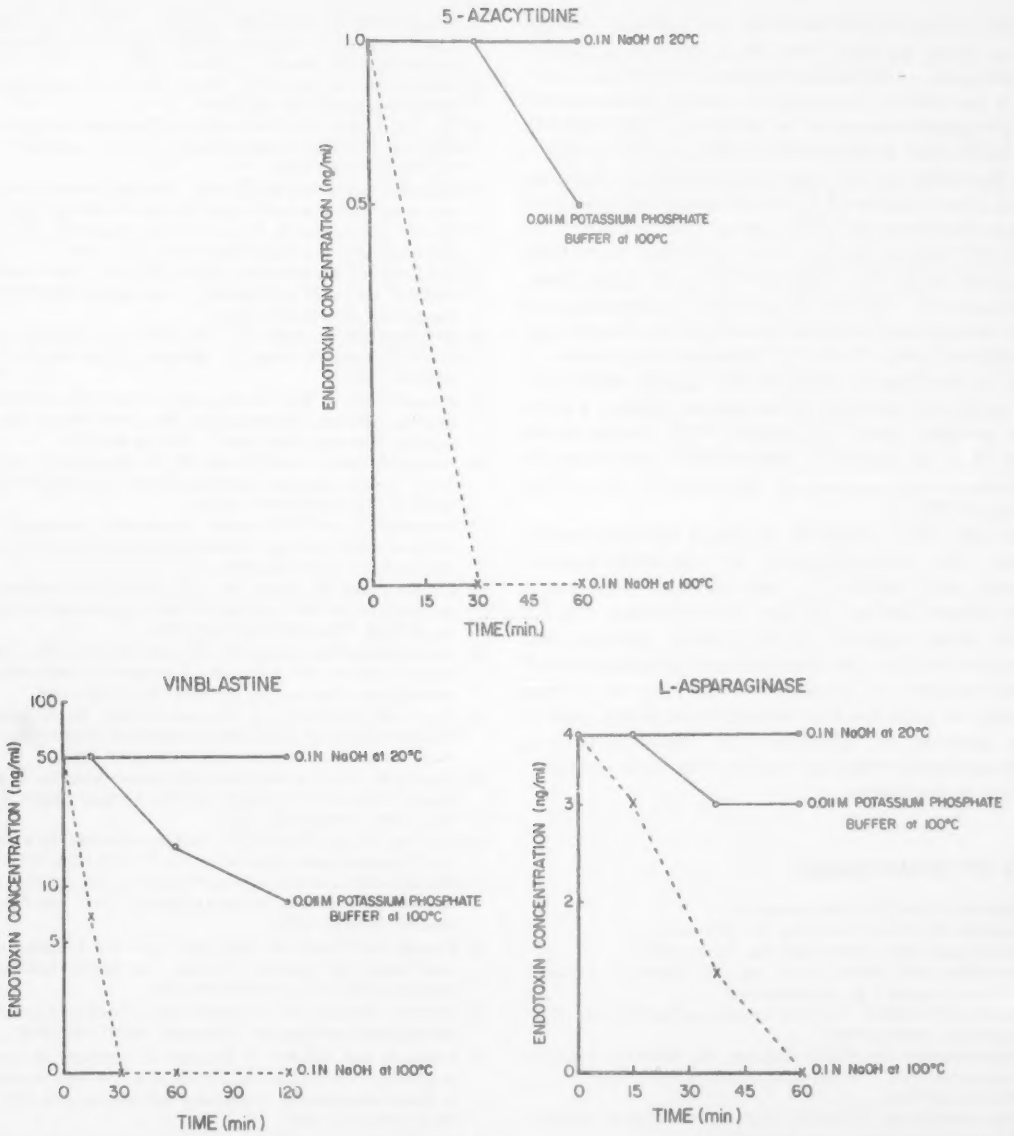

FIGURE 3.—Inactivation kinetics of *Limulus*-positive material in 5-azacytidine, vinblastine, and L-asparaginase. Endotoxin concentrations were determined by *Limulus* assay of serial twofold drug dilutions using the standard *E. coli* endotoxin curve.

dent rabbit pyrogen testing, although the *Limulus* assay was performed three times. The potential sources of error leading to such lack of correlation of these two assays include the following: *(a)* contamination of the drug with endotoxin during preparation for *Limulus* assay; *(b)* contamination of testing materials; *(c)* variable contamination of individual *vials* of a given lot of drug; or *(d)* variability in the standard endotoxin quantitation curves produced by different strains of *E. coli*. Scrupulous attention to sterile nonpyrogenic technique is obviously required to ensure the reliability of the *Limulus* assay and to avoid some of the sources of errors discussed above.

The clinical significance of the present findings is not clear at this time. In a variety of animal systems and in man, the repeated injection of endotoxin can induce a variety of clinical states as well as pyrogenic tolerance to endotoxin (24,28,34-36). Of particular importance to the present study is the fact that all of these phenomena are dose related. Most studies of the toxic sequelae have used endotoxin doses of 1-10 μg/kg and greater (18, 23,25,27). Many similar toxic symptoms have been observed after the administration of some chemotherapeutic agents (especially L-asparaginase and bleomycin), but the amount of endotoxin administered with clinically effective drug doses is only in the range of 0.0001-0.004 μg/kg, while 0.01-0.4 μg/kg of endotoxin is needed to produce a positive pyrogen test in rabbits (≥1°C temperature rise) (11). In contrast, immunologic tolerance to endotoxin can be shown at significantly lower dose levels (34,35).

At this time, however, a direct correlation between the contamination of chemotherapeutic agents with endotoxin and clinical phenomena must await further studies. Nevertheless, the *Limulus* assay appears to be a rapid, specific, and sensitive tool for the detection and quantitation of small amounts of bacterial endotoxin in a wide variety of parenterally administered drugs, and it may provide the pharmaceutic industry with a more accurate means of monitoring such contamination in the future.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053; dactinomycin
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-
Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin
DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-
Streptozotocin: NSC-85998
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate; Velban
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Use of an Elemental Diet in Animals During Treatment With 5-Fluorouracil (NSC-19893)[1,2,3]

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SUMMARY

Rats eating a diet containing casein hydrolysate (10% wt/wt) (diet 3) instead of whole casein (diet 1) exhibited increased tolerance to nine consecutive daily injections of 15 mg/kg of 5-fluorouracil (5-FU). The relative nutritional efficiency of diet 3 was significantly higher during 5-FU treatment. Serum albumin levels measured after 5-FU treatment dropped by only 2.7% in diet 3 groups and by 13.5% in diet 1 groups. Serum albumin values for rats on the control diet (Purina lab chow) were comparable to those on diet 1. No 5-FU-related mortality was observed in any of the groups. Intestinal brush border enzymes were determined in a group of rats on diet 1. At the end of 5-FU treatment statistically significant changes were observed: sucrase dropped to 30% of control and leucylnaphthylamide-hydrolyzing activity dropped to 19% of control. The activity of γ-glutamyltransferase did not change significantly. It is postulated that under these circumstances a mixture with a prevalence of free amino acids (casein hydrolysate) could be more readily absorbed than a corresponding mixture containing a larger proportion of oligopeptides.

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The pyrimidine analog, 5-fluorouracil (5-FU), is an established antineoplastic agent used in the control of several types of disseminated malignancies (1). Intestinal mucosal changes with associated changes in intestinal function are often a limiting factor to completion of therapy with this antimetabolite (2-5). The lesion originally involves proliferating as well as mature cells of the intestinal epithelium. The cells may appear vacuolated, flattened, or necrotic.

In previous studies it was demonstrated that the use of an elemental diet containing hydrolyzed casein instead of an equal amount of whole casein for 7 days before and after treatment partly protects the intestinal mucosa of rats against the lesions associated with a single ip dose of 400 mg/kg of 5-FU (6). However, despite an apparently favorable effect on the intestine, the elemental diet does not improve survival following a single ip dose of 500 mg/kg of 5-FU, although survivors on the elemental diet return to their original weight faster than controls (6). Similarly, when 5-FU was given in daily injections of 30 mg/kg on a 9-day schedule, the elemental diet failed to provide a favorable effect upon survival (G. Bounous, unpublished data). It became apparent that the systemic effects of the intestinal lesion are not clearly defined with high doses of the antimetabolite and that survival studies in rats do not represent the ideal experimental model to assess the possible systemic effects of clinical 5-FU-induced enteropathy. In the following experiments 5-FU is given in a standard clinical dose. The systemic nutritional effects of 5-FU enteropathy and its possible mitigation by dietary management are investigated.

MATERIAL AND METHODS

Male Wistar rats (225 g) were used in all experiments. The animals were kept in cages so constructed as to prevent coprophagy and contamination of food with feces. Water was allowed ad libi-

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tum; animals were housed in pairs. Diets utilized in this study were as follows: whole casein (diet 1), casein hydrolysate (diet 3), a combination of whole and hydrolyzed casein (diet 2), and control, Purina lab chow (diet 4). Specifics of each of the experimental diets are provided in table 1. The rationale for diet 2 is the occasional objection of patients to diet 3 (Flexical) because of the unpleasant taste of free amino acids. For this reason it was decided to investigate the effects of further reduction of the hydrolysate from 10% to 7% wt/wt with 3% wt/wt whole egg albumin replacing the corresponding amount of casein hydrolysate. This protein would also compensate for the relative deficiencies of methionine, cystine, phenylalanine, and tyrosine observed in casein hydrolysate (7). Indeed a 30% decrease in amino acid content makes diet 2 more palatable to humans than diet 3 (Flexical). Ten rats in each dietary group were given (ad libitum) either diets 1, 2, or 3 or Purina lab chow (diet 4) in

TABLE 1.—Composition of formula-defined diets

	g/100 g		
	Diet 1	Diet 2	Diet 3
Casein hydrolysate		8.800	12.560
Casein, edible grade	11.209	3,768*	
Sucrose	46.839	46.839	46.839
Corn syrup solids (glucose, oligosaccharides)	16.812	16.812	16.812
Soya oil, partially hydrogenated (long chain triglycerides)	11.982	11.982	11.982
Medium chain triglycerides	2.997	2.997	2.997
Tapioca starch	5.234	5.234	5.234
Flexical mineral premix†	2.611	2.611	2.611
Flexical vitamin-amino acids premix†	0.384	0.384	0.384
Choline chloride	0.028	0.028	0.028
Distilled water	1.00	0.553	0.553
Sodium citrate hydrous	0.656‡	0.656§	0.656§
Potassium chloride	0.248	0.248	0.248

*Egg albumin.

†Mead-Johnson Canada Ltd. To obtain 9.64 g% of protein or protein equivalent, mineral and water content of casein and casein hydrolysate were taken into account:

protein (or protein equivalent) = 9.64%

fat = 15%

carbohydrate = 68.8%

(sucrose 68%, mixture of glucose and glucose oligosaccharides 24.4%, starch 7.2%)

‡Added.

§Contained in casein hydrolysate.

powder form for 12 consecutive days. The caloric value of the three formula-defined diets was 4.4 kcal/g. Because of a variability in composition and fiber content, the exact caloric value of Purina lab chow cannot be calculated. However, considering a company-declared 24% wt/wt protein content and assuming a similar distribution of calories from fats and carbohydrates, the caloric value of this product could be estimated at a level comparable to the three formula-defined diets. Dietary intake and growth were recorded throughout the observation period, at the end of which the animals were killed and blood hematocrit and serum protein electrophoresis were determined. The data obtained in this control series were compared with those from another series identical to the former in terms of duration and type of dietary treatment. The only point of difference in the latter series was a daily ip administration of 5-FU[6] (15 mg/kg of body weight) given on the fourth to the 12th day of the diet for a total of nine consecutive injections. Each dietary group comprised 15 rats. Animals were killed 24 hours after the last injection of 5-FU.

A second series of experiments was designed to investigate the effect of 5-FU treatment on intestinal mucosal enzymes.

Control data for sucrase and peptidases (γ-glutamyltransferase and leucylnaphthylamide-hydrolyzing activity) were obtained in ten rats after 12 days on diet 1 (group A). A second group of ten rats (group B) ate diet 1 for 12 days and received ip injections of 5-FU (15 mg/kg of body weight) from the fourth day to the 12th day of the diet for a total of nine consecutive injections. The animals were killed 24 hours after the last injection of 5-FU. A third group of ten rats (group C) ate diet 1 for 12 days but beginning on the fourth day the daily amount of the diet was restricted for 9 days to match the lower dietary intake observed in rats during 5-FU treatment. Animals were killed in the morning with only 1 hour of prior fasting. Since rats eat little food in the early morning hours, the stomach was often found empty at the time of death. A short period of fasting was selected in order to avoid the effects of starvation upon intestinal mucosa. Immediately after decapitation, the small intestine from the pylorus to the ileocecal valve was removed in one piece. The resected intestines were then opened longitudinally and shaken vigorously in a large beaker of ice-cold saline. Mesenteric fat was removed, the segments were clamped at the four corners, and the mucosa was scraped off with the back of a scalpel over an ice-cold surface. The mucosa was homogenized in ice-cold saline (5 ml/g wet weight) in a Potter-Elvehjem homogenizer and centrifuged for 60 minutes at 105,000 × g at 4°C; the supernatant contained the cytoplasmic fraction. To prepare particulate fractions the pellet obtained by centrifuging a whole homogenate was washed twice in isotonic saline and then suspended in the same medium. Following each wash the pellet was recovered by centrifuging for 30 minutes at 105,000 × g (8).

Purified brush borders were prepared utilizing, without modifications, a technique developed for human brush border membranes (9). Sucrase activity was assayed according to a modification by Lloyd and Whelan (10) of Dahlqvist's method (11). γ-Glutamyltransferase was determined according to the method of Naftalin et al (12). Leucylnaphthylamide-hydrolyzing activity was determined according to the method of Goldbarg and Rutenburg (13). Proteins were determined by the biuret method and by the method of Lowry et al (14). Substrates for enzyme assay were obtained from Sigma Chemical Co, St. Louis, Mo.

Levels of statistical significance were determined by the student's unpaired t test. P values of < 0.05 were considered to be significant.

Electron Microscopic Methods

Animals on diet 1 were killed the morning after the end of the 9-day 5-FU schedule. The first part of the jejunum was fixed by injection of 3% distilled glutaraldehyde buffered with cacodylate 0.1 M in the lumen of the intestine. A piece of tissue was immediately removed and immersed in the same fixative solution for 30 minutes at 4°C. After a 2-hour fixation, the blocks were rinsed for 24 hours in the cacodylate buffer with 7.5% sucrose, post-fixed in 2% OsO_4 in the same buffer, dehydrated, and embedded in Epon. Ultrathin sections were stained with uranylacetate and lead citrate and examined in an EM 300 Philipps electron microscope.

RESULTS

Table 2 illustrates the effects of the four dietary regimens upon body weight under normal conditions and during 5-FU treatment. All animals survived the experiment and were killed 24 hours after the last injection of 5-FU. Despite the somewhat lower intake of elemental diets (diets 2 and 3) the weight gain per gram of food in the control series is almost identical in all dietary groups. Soon after the start of and throughout the 5-FU treatment all animals ate less in all dietary groups. However, the relative nutritional efficiency

[6]Sodium salt of 5-FU (pH 8.8).

TABLE 2.—Nutritional parameters in control and 5-FU-treated rats as a function of diet*

	Food intake† (g/rat/24 hrs)		Weight change† (g rat/g food/24 hrs)		Blood hematocrit (%)		Total serum protein (g/100 ml)		Serum albumin (g/100 ml)	
Diet	Control	5-FU	Control	5-FU	Control	5-FU	Control	5-FU	Control	5-FU
1	21.1±1.7	8.6±1.4	±0.32±0.02	−0.69±0.02	41.3±0.8	48.9±1.4	6.0±0.1	4.5±0.1	3.0±0.1	2.6±0.09
2	17.8±0.2	8.4±0.9	±0.31±0.02	−0.73±0.08	41.4±0.9	48.7±1.3	6.3±0.2	4.9±0.1	3.5±0.1	2.6±0.08
3	16.6±0.7	9.4±1.1	±0.30±0.01	−0.39±0.03	41.8±1.0	50±0.6	5.7±0.1	5.1±0.09	2.8±0.08	2.7±0.08
4	22.6±1.8	8±0.8	±0.31±0.02	−0.70±0.03	41.4±1.0	49.1±1.3	5.9±0.1	4.5±0.09	3±0.1	2.6±0.09

*Each value represents the mean ±SEM of data. See text for statistical significance of results.
†Food intake and weight changes are expressed as the average value for the 9-day study period.

of diet 3 as reflected by weight loss per gram of food eaten is significantly higher throughout 5-FU treatment. The data for diet 1 (whole casein) are very similar to those for Purina rat chow (diet 4).

During 5-FU treatment, diet 2 failed to provide any significant advantages over diet 1 or the control diet. The data for serum protein, reported in table 2, reflect the protective effect of diet 3 already manifested by a better preservation of lean tissue mass. The increase in blood hematocrit as a result of 5-FU treatment is similar in all dietary groups (table 2); therefore, differences in serum protein may be properly attributed to nutritional differences rather than to variations in intravascular fluid shift. In these studies, total serum protein and albumin values after 5-FU treatment are compared with corresponding values of control rats fed the same diets over the same period of time. Percent decreases on diet 3 were respectively 10% and 2.7% of control, and the corresponding figures are 22% and 24.7% for animals on diet 2 and 24.4% and 13.5% for animals on diet 1. The superior nutritional efficiency of diet 3 during 5-FU treatment is primarily reflected in the serum albumin value which is maintained at control levels throughout treatment, whereas a significant drop in serum proteins is observed at the end of 5-FU treatment in the other three dietary groups.

There is no significant difference between dietary groups with regard to control serum globulin levels; following 5-FU, the difference in globulin between diet groups is less striking than that of albumin.

Tables 3 and 4 illustrate the effects of 5-FU treatment and food restrictions upon tissue parameters and enzyme activity of the small bowel mucosa. In the pair-fed group (group C), the average daily intake of diet 1 was restricted to 8.5 ± 1 g (mean ± SE) in order to match the lower intake observed during 5-FU treatment. The average daily weight loss for the 9-day period was, in this group, 4.2 ± 0.2 g, somewhat less than the 6.2 ± 0.3 g loss observed on diet 1 plus 5-FU ($P < 0.05$). Food restriction alone appears to bring about some reduction in the wet weight of the intestinal mucosa, accompanied by a rise in protein concentration (table 3). Treatment with 5-FU appears to bring about only a decline in protein and DNA

TABLE 3.—Tissue parameters in 5-FU-treated rats (B), pair-fed rats (C), and control rats (A): all animals were fed diet 1*

Group	Mucosal wet weight (g)	Protein content (mg/ml)				DNA content (mg/ml): particulate fractions
		Homogenate	Cytosol	Particulate fractions	Brush borders	
A	2.04±0.13	10.24±0.29	5.59±0.21	36.34±5.49	3.10±0.47	5.77±0.63
B	2.13±0.21	6.03±0.68	2.77±0.29	20.75±2.27	4.15±0.43	2.95±0.34
C	1.54±0.06	16.58±0.83	8.07±0.33	58±6.33	5.36±0.48	7.36±1.53

*Each value represents the mean ± SEM of data from 10 rats. See text for statistical significance of results.

TABLE 4.—Enzyme activity (units/mg of protein) in 5-FU-treated rats (B), pair-fed rats (C), and control rats (A): all animals were fed diet 1

Enzyme	Brush borders*		
	A	B	C
γ-Glutamyl-transferase	0.14±0.01	0.14±0.009	0.12±0.01
Leucylnaphthylamide-hydrolyzing activity	0.06±0.003	0.01±0.004	0.03±0.003
Sucrase	0.36±0.04	0.11±0.01	0.16±0.02

*Each value represents the mean ± SEM of data from 10 rats. *See* text for statistical significance of results.

concentration without a change in the mucosal weight; no change in brush border proteins was observed.

The activity of γ-glutamyltransferase in the brush borders is essentially similar in the various groups. Sucrase and leucylnaphthylamide-hydrolyzing activities are significantly reduced following food restriction (group C) as compared to control values (group A). However, after 5-FU (group B), the activities for both enzymes are significantly lower than the corresponding values in the pair-fed animals (group C) (table 4). No apparent ultrastructural change was observed in the microvilli of small intestinal mucosa of rats fed diet 1 plus 5-FU for 9 days.

DISCUSSION

A diet containing hydrolyzed casein instead of intact casein shows normal nutritional efficiency under control conditions. However, during treatment with 5-FU the same diet exhibits a nutritional efficiency higher than control diets as reflected by body weight and serum albumin levels. It is somewhat puzzling to observe that substitution of as little as 30% of protein hydrolysate by an equivalent amount of high-quality protein abolished this desirable property (diet 2). Indeed, diet 2 showed an excellent nutritional value in control animals, with higher serum albumin levels per gram of food than any of the other diets. Moreover, tolerance to irradiation was found to be greater in rats eating diet 2 than in rats eating diet 1 or Purina (15). Reductions in the percent of hydrolysate (diet 2) do not significantly alter the intake of diet 2 as compared to diet 3 in control rats. During the first 10-12 days of the elemental diets, rats tend to eat somewhat less than normal. In the control series, in spite of the similar changes in weight per gram of food intake between the various dietary groups, those consuming the hydrolysate diet gain about 20% less than those on the nonhydrolysate diets. Subsequently, however, food intake and growth are quite comparable with values obtained with Purina and other nonhydrolyzed synthetic diets. Over a 30-day period, the average daily intake of food and the average growth of 150-g Wistar rats were 18.4 ± 1.7 g (mean ± SE) and 5.6 ± 0.8 g respectively in the Purina group. The corresponding values in the diet 3 group were 17.1 ± 0.7 g and 5.4 ± 0.3 g. For the sake of analogy with a conceivable clinical situation, 5-FU treatment was initiated after only 3-4 days of dietary management.

The positive effect of an elemental diet such as diet 3 during antimetabolite treatment has been confirmed by others in laboratory animals[6] as well as in cancer patients undergoing intensive chemotherapy. In the latter situation, studies at our institution (16) and at the M.D. Anderson Hospital in Houston[7] have shown that if patients eat diet 3 instead of conventional food the weight loss and intestinal symptoms associated with intensive cancer chemotherapy are minimized. Our experiments

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deal with clinical, not lethal doses of the antimetabolite. Our findings on mucosal weight and protein concentrations are somewhat puzzling in view of the apparent opposite direction of change for 5-FU-treated and pair-fed rats. The mucosal weight loss and the gain in mucosal protein and DNA content observed in group C might be attributed to water loss because of dietary restriction. In keeping with this hypothesis are the observations of McManus et al (17) who, after total starvation in rats, observed a decrease in the mucosal wet weight whereas mucosal dry weight remained unchanged. On the other hand, the preservation of mucosal wet weight with significant reduction of cellular components (proteins, DNA) observed in group B could be the result of water retention and edema produced by a potent enterotoxic drug such as 5-FU.

Two of the best known brush border "digestive" enzymes were chosen for this study in order to correlate the effects of 5-FU upon the intestinal mucosa with dietary protection. Brush border aminopeptidases and disaccharidases are thought to be involved in digestion of dietary proteins and disaccharides because they have been shown to be significantly reduced during starvation and to adapt to dietary changes (18). γ-Glutamyltransferase was selected as an enzyme thought to be involved in amino acid transport through the γ-glutamyl cycle (19).

It is noteworthy that the activity of the latter enzyme appears unchanged following food restriction or 5-FU treatment, whereas the marked decrease in brush border sucrase and leucylnaphthylamide-hydrolyzing activities observed following 5-FU significantly exceeds the drop related to reduced food intake (table 4). The experimental data presented here show that an important protective element in the diet is the substitution of protein by hydrolysate. The protein hydrolysate in diet 3 contains about 66% nitrogen in the form of free amino acids, the remaining being in the form of oligopeptides (20). The observed threefold reduction in brush border peptidase activity may interfere with the terminal digestion of oligopeptide and hence prevent the transport of dipeptides towards the cytosol. Moreover, it is conceivable that 5-FU may adversely affect the synthesis of pancreatic hydrolases. Under these circumstances a mixture with a prevalence of free amino acids could be more suitable to absorption than a corresponding mixture containing a larger proportion of oligopeptides.

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Development of Resistance to Combinations of Six Antimetabolites in Mice With L1210 Leukemia[1,2,3]

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SUMMARY

The development of resistance to combinations of 6-mercaptopurine, 6-thioguanine, 6-methylmercaptopurine riboside, methotrexate, 5-fluorouracil, and cytosine arabinoside was studied in L1210 leukemia through 60 transfer generations. The treatment schedules were either simultaneous or offset. In simultaneous administration, one sixth of the LD10 of each of the six drugs was administered within a few minutes, daily for 6 days. In offset administration, the drugs were given either in the above-listed order or in reversed order, with one drug given each day. In the simultaneous combination treatment protocol 31 transfer generations were necessary to reach partial resistance, but in the two offset combination schedules only five and three generations were needed. The relative rate of development of resistance to the individual drugs was slower in the three combination schedules than in single-drug schedules. Resistance to 6-mercaptopurine and 6-thioguanine was completed after four generations on the offset combination schedules, but only after 28 generations on the simultaneous schedule.

[Cancer Treat Rep 60:23-27, 1976]

The potential circumvention of drug resistance and the consideration that by the use of drugs of different mechanisms additive or synergistic antitumor effect and less than additive host toxicity might be obtained, were factors which prompted studies of combination drug treatment (1-6). Surprisingly, the development of drug resistance to combination therapy has received little experimental attention. A delay in the onset of resistance was noted if methotrexate and a terephthalanilide were used in combination (1). When L-asparaginase, methotrexate, and cytosine arabinoside were administered simultaneously to L5178Y leukemia-bearing mice, the development of resistance to this combination took longer than the development of resistance to each drug alone (7).

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[3]All drugs were supplied by Drug Research and Development Program, Division of Cancer Treatment, NCI. L1210 leukemia was maintained in mice obtained from A. R. Schmidt Co, Madison, Wis.

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The purpose of this study was to compare the rates of development of resistance to combination and single-drug treatments and to attempt to delay or prevent the emergence of resistance by means of various treatment schedules.

Most of our experiments on the resistance and cross resistance to single agents have been done with the mouse L1210 leukemia (8,9). Therefore, we also used L1210 to study the development of resistance to combination chemotherapy. In the study of combination therapy there are so many possible combinations and treatment schedules that, unless handled systematically, it is very difficult to compare and detect the factors responsible for the results. Therefore, we selected drug combinations and schedules which are suited for basic and systematic studies of the development of resistance to combination therapy.

MATERIALS AND METHODS

L1210 leukemia was maintained in BDF_1 (C57BL/6 × DBA/2) mice. The treated and control groups usually consisted of five or ten male mice weighing 19-24 g.

6-Mercaptopurine, 6-thioguanine, 6-methylmercaptopurine riboside, methotrexate, 5-fluorouracil

(5-FU), and cytosine arabinoside were used. The treatment was always the same; one injection daily for 6 days, starting 1 day after ip inoculation of 10^6 cells. A 6-day injection schedule was chosen because the survival time of BDF_1 mice injected ip with 10^6 L1210 cells is 7 or 8 days. With this schedule the LD10s for each drug were determined by plotting the dose-mortality data (which were obtained for several dose levels with ten normal BDF_1 mice each) on probability paper. The LD10 is defined as the dose that kills 10% of the animals by 4 weeks after the start of treatment (10).

The treatment schedules were either simultaneous or offset. In simultaneous administration, one sixth of the LD10 of each of the six drugs was administered within a few minutes, daily for 6 days. In offset administration, the drugs were given according to schedule 1:

Day 1, LD10 of 6-mercaptopurine
Day 2, LD10 of 6-thioguanine
Day 3, LD10 of 6-methylmercaptopurine riboside
Day 4, LD10 of methotrexate
Day 5, LD10 of 5-FU
Day 6, LD10 of cytosine arabinoside;

or according to schedule 2:

Day 1, LD10 of cytosine arabinoside
Day 2, LD10 of 5-FU
Day 3, LD10 of methotrexate
Day 4, LD10 of 6-methylmercaptopurine riboside
Day 5, LD10 of 6-thioguanine
Day 6, LD10 of 6-mercaptopurine.

The sublines treated by a combination of drugs were checked after every four treatment generations against each of the individual drugs to determine the relative speed of the development of resistance to the drugs.

RESULTS

The LD10s for the six compounds in normal mice treated ip daily for 6 days are listed in table 1. The effects of these doses on L1210 tumor-bearing mice were quite similar, the median survival times (MSTs) ranged from 13 to 15 days, versus 8 days for the controls.

A combination containing one sixth of the LD10 of each of the six compounds was less toxic than the full LD10s of the single agents (tables 1 and 2). Also, whereas twice the LD10s of the single agents killed almost all mice, the double doses of all three combination schedules were less toxic; eg, the double schedule 2 offset dose caused no deaths. These results show that the combined drugs caused less than additive host toxicity. However, in contrast, all three combination schedules caused a greater increase in the survival time of L1210 tumor-bearing mice than any of the six individual agents.

The number of treatment generations necessary for the development of complete resistance to the LD10s ranged from one for 6-mercaptopurine and

TABLE 1.—Effect of 6 antimetabolites on L1210 leukemia

Drug	LD10* (mg/kg)	No. of mice	Weight change on Day 7 (g)	MST ± SE† (days)
6-Mercaptopurine	56	25	-2.2	14 ± 0.5
6-Thioguanine	2.9	20	-2.4	13 ± 0.2
6-Methylmercapto-purine riboside	32	20	-3.0	14 ± 0.4
Methotrexate	2.4	15	-2.9	15 ± 0.4
5-FU	28	20	-3.2	15 ± 0.3
Cytosine arabino-side	60	20	-2.1	15 ± 0.3
Control	0	20	+1.5	8 ± 0.2

*Six daily ip injections were given starting 1 day after ip inoculation of 10^6 L1210 cells; twice the LD10 killed all 10 mice in each group, with the exception of the cytosine arabinoside group (6 of 10 mice).

†The MSTs of all treated groups differed significantly ($P < 0.01$) from that of the controls.

TABLE 2.—Toxicity and antitumor effect of 3 combination schedules with 6-mercaptopurine, 6-thioguanine, 6-methylmercaptopurine riboside, methotrexate, 5-FU, and cytosine arabinoside

Schedule	LD10*	No. of normal mice/ mortality (%)	L1210 tumor-bearing mice		
			No.	Weight change on Day 7 (g)	MST ± SE (days)
Simultaneous	× 1	20/0	35	-1.9	18 ± 0.3
	× 2	25/68	25	-4.3	9 ± 1.6
	× 3	10/100	5	-4.8	8 ± 0.9
Schedule 1 offset*	× 1	20/0	45	-1.6	17 ± 0.5
	× 2	15/40	25	-2.9	15 ± 0.6
	× 3	10/100	5	-4.4	8 ± 0.9
Schedule 2 offset*	× 1	20/0	45	-1.7	16 ± 0.5
	× 2	15/0	25	-2.7	14 ± 0.7
	× 3	10/80	5	-4.0	8 ± 1.3
Control	0		65	+1.3	8 ± 0.1

*Treatment was started 1 day after ip inoculation of 10^6 L1210 cells and was given in 6 daily injections as single, double, or triple LD10s.

TABLE 3.—Development of resistance to single-drug treatment in L1210 leukemia at 2 dose levels

Treatment*		No. of generations necessary for development of complete resistance to the—	
Drug	Dose level	1/6 LD10	LD10
6-Mercaptopurine	1/6 LD10	1	2
	LD10		1
6-Thioguanine	1/6 LD10	1	2
	LD10		1
6-Methylmercapto-purine riboside	1/6 LD10	3	5
	LD10		3
Methotrexate	1/6 LD10	5	10
	LD10		5
5-FU	1/6 LD10	7	12
	LD10		6
Cytosine arabino-side	1/6 LD10	4	5
	LD10		5

*Six daily ip injections were given starting 1 day after ip injection of 10^6 cells; there were 5-10 male BDF_1 mice/group.

6-thioguanine to five for methotrexate and six for 5-FU (table 3). For the sixth part of the same dose it took, in general, longer to produce the same degree of resistance; it took ten generations for methotrexate and 12 for 5-FU.

The patterns of resistance development for the three combination schedules were different (fig 1). In simultaneous treatment, it took 31 treated generations to reach partial resistance, ie, to reduce the MST from 18 days in the first generation to 11 days. In the meantime, the survival time of the controls increased from 8 to 9 days. In contrast, the same degree of resistance was achieved by the fifth generation in the offset schedule 1 group and by the third generation in the offset schedule 2 group, in both instances much faster than in the simultaneous group. Afterwards, as is shown in figure 1, the survival times in all three combination schedules fluctuated around 11 days until the end of the experiments at generation 61.

The behavior of the MSTs of the untreated control groups is also of interest. From the baseline of 8 days, MSTs increased in the two offset groups to 9 days and in the simultaneous treatment group to 11 days. Thus at generation 61 the MSTs in the two offset groups were 11 days for the treated groups and 9 days for the control groups, and the respective MSTs in the simultaneous group were both 11 days.

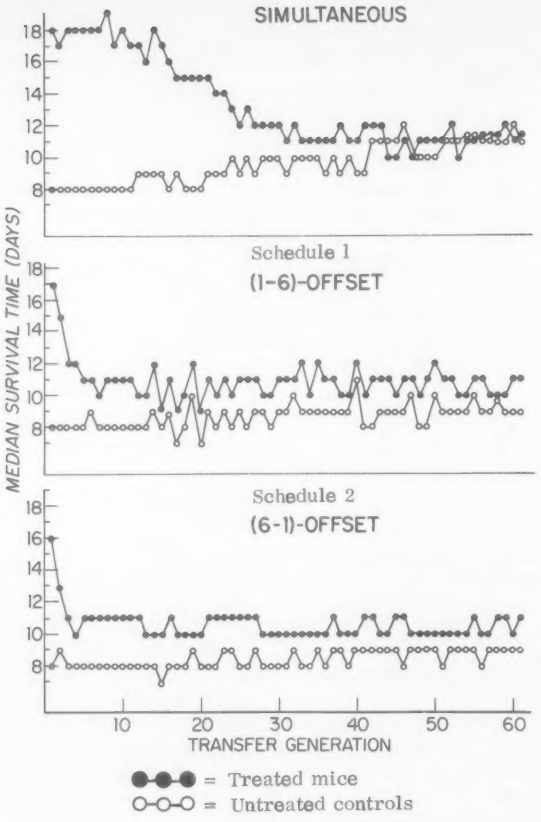

FIGURE 1.—Effect of 3 different combination treatment schedules (simultaneous, [1-6]-offset [schedule 1], [6-1]-offset [schedule 2]) on the development of resistance in mice with L1210 leukemia.

After every four treatment generations, the three sublines treated by the combination of the six drugs were tested against each of the individual combination drugs. Resistance to 6-mercaptopurine and 6-thioguanine when used as single drugs was complete in generation 2 (tables 3 and 4). Complete resistance to 6-mercaptopurine and 6-thioguanine was also present in the two offset combinations at their first test in generation 4. However, in simultaneous treatment, 28 generations were required. For 6-methylmercaptopurine riboside administered as a single drug only five generations were needed, but it took 24 generations for the schedule 2 offset, 28 for the simultaneous, and > 60 for the schedule 1 offset combination. Methotrexate and 5-FU produced resistant sublines after ten and 12 generations, respectively, but in all three combinations, resistance to neither compound was completed during 60 generations.

TABLE 4.—No. of transfer generations necessary to develop complete resistance to 6 antimetabolites when used in 3 different combination schedules*

Drugs	No. of generations with—		
	Simultaneous schedule	Offset schedule 1	Offset schedule 2
6-Mercaptopurine	28	4	4
6-Thioguanine	28	4	4
6-Methylmercaptopurine riboside	28	> 60	24
Methotrexate	> 60	> 60	> 60
5-FU	> 60	> 60	> 60
Cytosine arabinoside	28	52	56

*The LD10s were given ip in 6 daily injections starting 1 day after ip inoculation of 10^6 cells from the treated group of the previous generation. After every 4 treated generations, the sublines were tested against the LD10 of each drug; there were 5-10 male BDF_1 mice/group.

Also, the development of resistance to cytosine arabinoside took much longer with combination treatment than by use of cytosine arabinoside alone.

DISCUSSION

The development of resistance to antimetabolites is usually due to a decrease or increase in the activity of enzymes involved in the activation of the antagonists and/or to changes related to the transport of the drugs into the cells (11,12). The resistance to a single drug is usually associated with multiple enzyme changes. It is, therefore, almost impossible to anticipate the great variety of interactions possible between enzymes or enzyme systems in response to treatment of cells with a combination of drugs having different mechanisms of action. Here the conditions for the development of resistance are obviously much less likely to be met than those for the development of resistance to one drug.

From the results of this experiment it is apparent that the development of resistance was much slower to combined drug treatment, especially with the simultaneous schedule, than if the agents were used singly. The development of resistance determined the outcome of treatment.

Of the six agents we used, 6-mercaptopurine- and 6-thioguanine-resistant lines usually (but not always [13]) show complete cross resistance to the

related purine analogs. Both purine analogs were selected in order to study their combined effect on the development of resistance to combination therapy. They rapidly produced complete resistance if used singly or in the two offset combinations. The rapidity of development of resistance appears to be compatible with a simple selection mechanism. However, the question arises, why did the development of resistance to 6-mercaptopurine and 6-thioguanine in the simultaneous treatment schedule take 28 treatment generations? It appears that the rapid development of resistance of these two drugs in both offset schedules was at least partly responsible for the relatively fast decline in the chemotherapeutic effect of the two offset combinations and the fast production of resistance, as compared with the simultaneous combination schedule. The development of resistance to the other four agents was similar both in the simultaneous and the offset treatments.

Presently, acquired drug resistance is the major limitation of cancer chemotherapy. Our results with L1210 leukemia show that the rate of development of resistance to combination therapy varies greatly, depending on the way these drugs are combined and especially on the schedule of treatment. The common view is that acquired drug resistance is due to gene or chromosomal mutation and is, therefore, irreversible. Recent evidence (14-17) suggests instead epigenetic changes, which in association with the epigenetic instability of the tumor cell in general (18-21) indicates the possibility of reversal. Therefore, the influence of various selected treatment schedules and drug combinations on the prevention and reversal of acquired drug resistance should be investigated in more detail.

LIST OF COMPOUNDS

L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

6-Methylmercaptopurine riboside: NSC-40774; CAS reg. No. 7143-67-1; 9*H*-purine, 6-(methylthio)-9-β-D-ribofuranosyl-, dihydrate

6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-

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5-Fluorouracil (NSC-19893), DTIC (NSC-45388), BCNU (NSC-409962), and Vincristine (NSC-67574) in Advanced Colorectal Carcinoma[1,2]

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SUMMARY

Thirty-three patients with advanced colorectal carcinoma were treated with 5-day courses of 5-fluorouracil (10 mg/kg/day, iv, Days 1-5), DTIC (3 mg/kg/day, iv, Days 1 and 2), BCNU (1.5 mg/kg/day, iv, Day 1 only), and vincristine (0.025 mg/kg/day, iv, Day 1 only) at 5-week intervals. One patient achieved CR and six patients achieved PRs. Five of ten patients treated within 8 weeks after detection of advanced cancer and two of 23 patients treated more than 8 weeks after detection of advanced cancer had CR or PR ($P = 0.02$). After most courses toxicity was moderate, although it necessitated a change to another regimen for one patient. One patient refused further cancer chemotherapy and one patient died due to severe bone-marrow toxicity.

[Cancer Treat Rep 60:29-32, 1976]

In a preliminary report on treatment with 5-fluorouracil (5-FU), DTIC, BCNU, and vincristine, van Eden et al (1) observed CR or PR in seven of 15 patients with colorectal cancer. In a randomized study of colon cancer, Falkson et al reported CR or PR in 12 of 28 patients who received combined therapy and in six of 24 patients who received 5-FU alone (2). Based on these results, combined therapy may be superior to single-drug treatment ($P = 0.145$). This study was undertaken to continue the evaluation of combined therapy in advanced colorectal carcinoma.

MATERIAL AND METHODS

Patients

Thirty-three patients with advanced colorectal carcinoma were admitted to Finseninstitutet between October 1972 and December 1974 and received combined therapy.

All of the patients had histologically proven primary tumors. Fifteen patients had colon carcinoma and 18 had rectal carcinoma. There were 15 men and 18 women. Eight patients had recurrent cancer and eight had metastatic cancer. Of 17 patients with local lesions, 12 had received palliative resections and five had inoperable disease. Recurrent or metastatic cancer was confirmed by biopsy in ten patients and was assumed, through clinical judgment, in six. Five patients had received radiation treatment for local lesions less than 3 months before the combined therapy but had other measurable lesions which had not received irradiation to serve as indicators of response to the combined therapy. Thirty patients had received no prior cancer chemotherapy before the combined therapy. Two patients had received therapy with cyclophosphamide and one with methotrexate. None of the patients had had surgical treatment within 10 days before the combined therapy. All patients had measurable lesions.

When treatment was started, all patients had white blood cell (wbc) counts greater than 5000 cells/mm^3 and platelet counts greater than 100,000 platelets/mm^3. Six patients had slightly elevated serum creatinine. Twenty-four patients were up and about for more than 50% of their waking hours (group 1). Nine patients were confined to their beds for more than 50% of their waking hours (group 2). The median age was 59 years (range, 35-80 years).

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[2]5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU.

DTIC: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide.

BCNU: CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

Vincristine: CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

DTIC and BCNU were supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute.

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Patient Evaluation and Followup

Before each course of treatment, clinical examination, wbc and platelet counts, hemoglobin level, and chest roentgenogram were obtained for all patients. Serum creatinine, serum alkaline phosphatase, serum glutamic oxaloacetic transaminase, bilirubin, protein, albumin, calcium, uric acid, reticulocyte count, and leukocyte differential determinations were obtained on the 1st and 12th days. During the course, the hemoglobin level and leukocyte and platelet counts were obtained each day before the treatment and weekly in the interval between the courses. Eleven patients had repeated liver scans. Postmortem examination of 20 of 28 patients who died during the study was performed.

Drug Administration and Dose Schedule

The patients received 5-day courses at 5-week intervals. The treatment schedule was 5-FU (10 mg/kg/day, iv, Days 1-5), DTIC (3 mg/kg/day, iv, Days 1 and 2), BCNU (1.5 mg/kg/day, iv, Day 1 only), and vincristine (0.025 mg/kg/day, iv, Day 1 only) via bolus injection. We gave metoclopramide (Metoclol, Moriperan, Primperan) or chlorpromazine (Thorazine) before treatment to inhibit vomiting. New courses were given until progressive disease was detected.

Assessment of Response

CR was defined as the disappearance of any tumor lesion and cancer symptom. PR was defined as at least a 50% regression of the product of the greatest length and width of a representative tumor lesion and no sign of progression. Improvement was defined as a regression of 25%-50% of a calculated tumor area. No response was defined as changes in a calculated tumor area limited to ± 25%. Progression was defined as tumor enlargement of 25% or more in any calculated tumor area or the appearance of a new tumor. No lesion treated with local radiation served as an indicator of remission to drugs. Duration of response was assessed as being the period from the 1st day of response until progression was noted.

All patients who died within two courses of treatment were considered to be treatment failures (TF). Patients with measurable lesions who refused to continue treatment were also considered TF.

RESULTS

Therapeutic Response

One patient had CR and six patients had PR. Response lasted for a median of 2.4 months with a range of 1.4-14.3+ months. Four of the responders had colon carcinoma and three had rectal carcinoma. One of the responders had pulmonary lesions, two had hepatic lesions, three had intra-abdominal masses, and one had a perineal carcinomatous fistula. The evaluated lesions had been histologically proven in three patients with PR. Three patients had no change, 11 had progressive disease, and 12 were TF. Of the 12 TF patients, seven died within two courses of treatment and five refused further treatment.

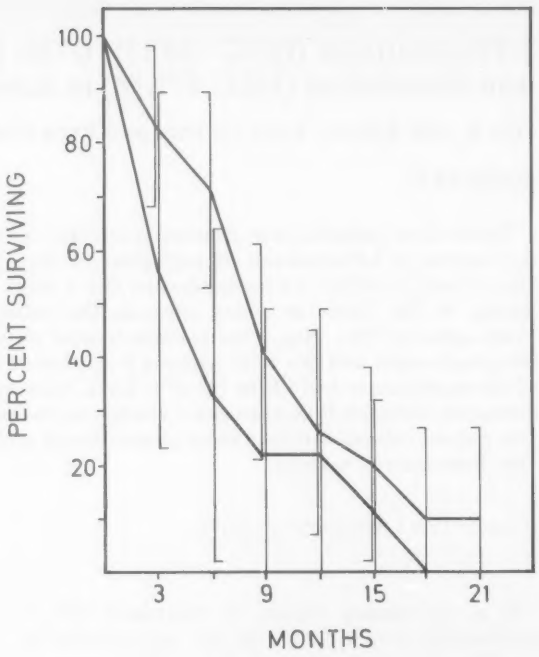

FIGURE 1.—Survival time ± 2 SDs from the beginning of therapy. Upper line = 24 patients who were up and about for more than 50% of their waking hours; lower line = 9 patients confined to their beds for more than 50% of their waking hours.

Five of ten patients treated within 8 weeks after detection of advanced cancer and two of 23 patients treated more than 8 weeks after detection of advanced cancer had CR or PR. The response rate was significantly different ($P = 0.02$).

Six patients in group 1 and one patient in group 2 had CR or PR ($P = 0.36$).

The survival was significantly superior ($P = 0.01$) for group 1 as compared to group 2, 5 months from the start of treatment (fig 1).

Toxicity

Figures 2 and 3 show the hematologic toxic effects after the first course of the combined thera-

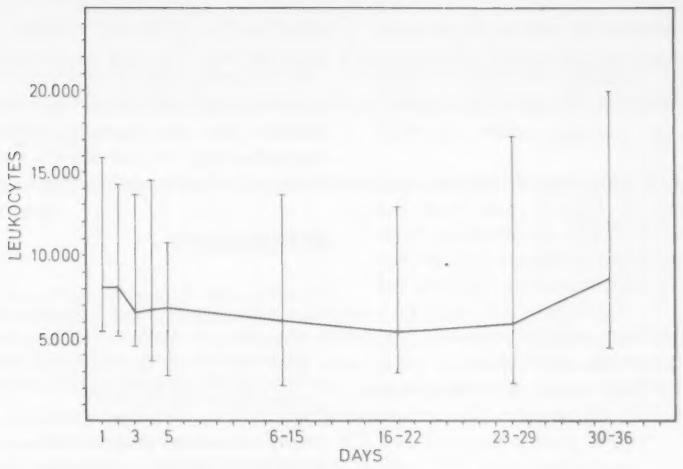

FIGURE 2.—Range and median of wbc after initial course of combined therapy.

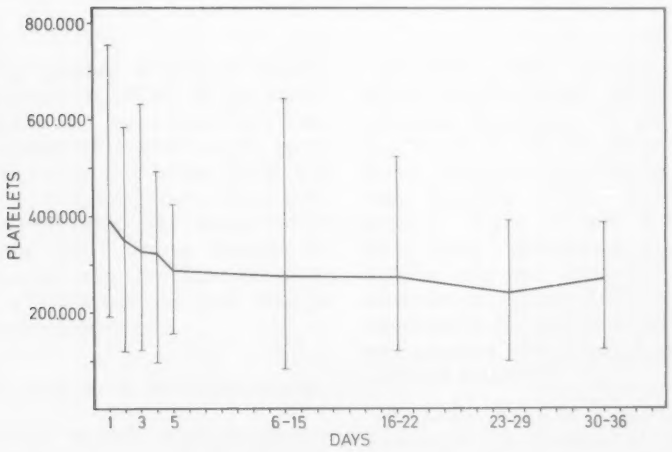

FIGURE 3.—Range and median of platelet count after initial course of combined therapy.

py. One patient died because of extreme bone-marrow toxicity. This patient had bone-marrow carcinosis. He received the combined therapy 3 days after receiving pelvic irradiation and had thrombocytopenia with a nadir platelet count of 8000 platelets/mm^3.

Twenty-eight patients experienced nausea and vomiting most severely on Days 1 and 2 of the treatment. One patient suffered so severely from vomiting that it necessitated a change to another regimen. Three patients experienced nausea but did not vomit.

Nine patients had diarrhea, eight had stomatitis, and four had severe alopecia, necessitating wigs. One patient refused further cancer chemotherapy due to the toxic effects.

DISCUSSION

The overall response to the combined therapy in advanced colorectal carcinoma in this study (seven of 33 patients) was lower than the results published so far (1,2) and corresponds to the overall response to 5-FU used as a single agent (3).

The performance status of the patients is a valuable tool for estimation of prognosis. Our results indicate that the highest response rate in cancer chemotherapy in advanced colorectal cancer is found in patients with recently detected lesions.

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Comparative Evaluation of Three Combination Regimens for Advanced Malignant Melanoma: Results of an International Cooperative Study[1]

Writing Committee:[2] Gianni Beretta,* Gianni Bonadonna, Natale Cascinelli, Alberto Morabito, and Umberto Veronesi

SUMMARY

The therapeutic results of a controlled study with three multiple-drug regimens (regimen A: DTIC, vincristine, BCNU; regimen B: DTIC, vincristine, hydroxyurea; and regimen C: DTIC, actinomycin D, BCNU) in a total of 274 evaluable patients with advanced malignant melanoma are reported. CRs were significantly more frequent ($P < 0.01$) in regimens A (9.3%) and C (16.4%) compared with regimen B (1.1%). No significant difference in terms of CR plus PR was detected among the three regimens. In all regimens a higher number of CRs plus PRs was seen in patients with soft tissue metastases only, compared with those who had visceral involvement. In all three regimens patients achieving CR showed a longer duration of response and survival in comparison with patients achieving PR. The incidence of brain metastases was neither lowered nor delayed by the presence of BCNU in regimens A and C.

[Cancer Treat Rep 60:33-40, 1976]

Since 1970 a large number of clinical studies have confirmed the value of DTIC[3] in the treatment of advanced malignant melanoma. DTIC represents the most extensively studied single agent in melanoma, and its activity in terms of CR and good PR is generally recognized to be about 20% (1,2). Other conventional drugs are considered to be less effective than DTIC, mainly because regardless of the response rate they have failed to show any evidence of improved survival time in responders versus nonresponders.

In recent years a variety of drug combinations which usually include DTIC have been tried in advanced melanoma. So far, none of the treatments tested can be considered the regimen of choice. Furthermore, although the three-drug regimen including DTIC, BCNU, and vincristine[4] showed a higher number of responses in patients with visceral metastases compared to DTIC alone (3), the only two controlled studies comparing a combination against DTIC alone failed to show a superiority for the combination in terms of response rates, durations of responses, and median survival times (2).

In 1971 the WHO Collaborating Centres for Evaluation of Methods of Diagnosis and Treatment of Melanoma designed a controlled trial with the intention of comparing four three-drug combinations which all included DTIC. The purpose of the study was: (*a*) to determine the most useful combination; and (*b*) to determine the role of BCNU in decreasing the incidence of cerebral metastases.

[1]Received May 28, 1975; revised Oct 20, 1975; accepted Oct 22, 1975.

[2]This study was performed by the World Health Organization (WHO) Centres for Evaluation of Methods of Diagnosis and Treatment of Melanoma. The following institutions and investigators participated in the study: University of Sydney, Australia (*G.W. Milton*); Het Nederlands Kankerinstituut, Amsterdam (*P. Rumke*); Hospital A. Camargo, Sao Paolo, Brazil (*D.C. Bandiera*); Institute of Experimental and Clinical Oncology, Academy of Medical Sciences, Moscow, USSR (*A. Garin* and *M. Lechenizer*); Institut Jules Bordet, Brussels, Belgium (*Y. Kenis*); Istituto Nazionale Tumori, Milan, Italy (*E. Bajetta, G. Beretta,* and *G. Bonadonna*); Oncological Institute, Cracow, Poland (*K. Szczygiel*); and Oncological Institute, Brno, Czechoslovakia (*Z. Mechl*).

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[3]DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-.

[4]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

MATERIAL AND METHODS

Patient Population

A total of 463 patients with a histologically proven diagnosis of malignant melanoma were entered into the study from January 1971 to October 1974. In addition to physical examination, the presence of advanced disease was assessed through a number of radiologic and radioisotopic examinations. Occasionally, the histologic confirmation of clinical findings was obtained. No attempt was made to confirm the cause of a positive or suspicious liver scan by needle biopsy. Usually, patients with a performance status < 40 were excluded from the study. The median age was 46 years (range, 17-77 years). The ratio of men to women was 0.8:1.

Drug Administration

Table 1 outlines the treatments and schedules. DTIC, BCNU, and actinomycin D[5] were given by rapid iv injection while hydroxyurea[6] was given orally. In the absence of myelosuppression, therapy was usually repeated on Day 29. A dose-reduction schedule with a 50% decrease in the amount of each drug was carried out in the presence of a leukocyte count fall ranging from 3999 to 2000 cells/mm^3 and/or a platelet count fall ranging from 129,000 to 80,000 cells/mm^3. Below these levels drug administration was delayed until full bone marrow recovery was achieved. Treatment was continued until either progression or relapse after an initial response was evident. In the patients with a CR a total of 12 courses were given before treatment was discontinued. The same combination was resumed when a relapse occurred.

Adequacy of Treatment and Evaluable Groups

In this study drug administration was considered adequate if treatment was given for a minimum of two courses or if therapy resulted in tumor regression after fewer than two courses. Following these criteria, data on 166 of 463 patients (35.8%) were discarded because of inadequate treatment or for protocol violations (concomitant radiotherapy, lack of measurable parameters for drug response, or prolonged interval between two successive courses). A total of 297 patients were therefore considered for evaluation of the therapeutic response.

[5]Actinomycin D: NSC-3053.

[6]Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1.

Each center was assigned two regimens which were randomly allocated to all patients entering into the study. Unfortunately, not all centers initially joining the study could provide the expected number of patients and therefore, the four regimens became numerically unbalanced. The imbalance was further exaggerated due to the adoption of the above-mentioned criteria for adequate treatment. Therefore, it was decided to delete from the comparative evaluation the patients who were entered into regimen D (DTIC, actinomycin D, hydroxyurea) because of their limited number (23 evaluable patients). The analysis was conducted on a total of 274 patients. Forty patients (14.6%) were previously treated with single-agent chemotherapy but they were given drugs not included in the combination regimen.

Response Categories

The criteria for evaluating the degree of drug response were as follows:

CR.—Disappearance of all signs and symptoms of disease including recalcification of osteolytic metastases and the return to normal of all abnormal biochemical tests for a minimum of 1 month.

PR.—A > 50% decrease in the sum of the products of the two longest diameters of all tumors present for a minimum of 1 month.

Objective improvement.—A 25%-50% decrease in the longest perpendicular diameter of all lesions for at least 1 month. No new or advancing lesions were present in determining response.

TABLE 1.—Outline of combinations and schedules*

Regimen	Drug combination	Dose schedule
A	DTIC	100 mg/m^2, Days 1-5
	Vincristine	1.4 mg/m^2, Days 1 and 15
	BCNU	100 mg/m^2, Day 1
B	DTIC	100 mg/m^2, Days 1-5
	Vincristine	1.4 mg/m^2, Days 1 and 15
	Hydroxyurea	10 mg/kg, Days 7,10,13,17,21,24
C	DTIC	100 mg/m^2, Days 1-5
	Actinomycin D	0.05 mg/kg, Days 1 and 15
	BCNU	100 mg/m^2, Day 1
D	DTIC	100 mg/m^2, Days 1-15
	Actinomycin D	0.05 mg/kg, Days 1 and 15
	Hydroxyurea	10 mg/kg, Days 7,10,13,17,21,24

*Treatments were usually repeated on Day 29. All drugs were given iv except for hydroxyurea which was given orally q8h.

TABLE 2.—Percent comparison of response

Type of response	Regimen* A E (207)	A AT (129)	B E (122)	B AT (84)	C E (98)	C AT (61)
CR	5.8	9.3	0.8	1.1	10.2	16.4
PR	7.2	11.6	13.1	19	9.2	14.7
PR + CR	13	20.9	13.9	20.2	19.4	31.1
Overall	27.1	43.4	27.8	40.4	32.7	52.5

*E = entered; AT = adequately treated. No. in parentheses = No. of patients.

TABLE 3.—CR plus PR related to site of metastases

Site	No. of CR + PR/No. with metastases: Regimen A	Regimen B	Regimen C
Skin	18/83 (21.6%)	14/55 (25.4%)	17/47 (36.1%)
Lymph nodes	15/61 (24.5%)	11/44 (25%)	6/28 (21.4%)
Lung	8/40 (20%)	3/29 (10.3%)	2/13 (15.3%)
Liver	2/20 (10%)	1/9 (11.1%)	0/6
Bone	0/9	0/5	0/3
Brain	0/6	0/2	1/4 (25%)
Other	5/23 (21.7%)	3/15 (20%)	1/8 (12.5%)
Soft tissues only	15/51 (29.4%)	11/38 (28.9%)	15/33 (45.4%)
Viscera ± soft tissue	12/78 (15.3%)	6/46 (8.7)	4/27 (14.8%)

RESULTS

The comparative responses among the three evaluable regimens are reported in table 2. For adequately treated patients there was no significant difference in terms of incidence of CR between regimens A and C while both were superior to regimen B ($P < 0.01$). However, in terms of incidence of CR plus PR there was no significant difference among the three regimens. The percent difference in the response rate between adequately treated and all entered patients is evident. Figure 1 shows that considering CR plus PR, the duration of response was longer in regimen C compared to the other regimens. However, the difference was not statistically significant ($P > 0.05$).

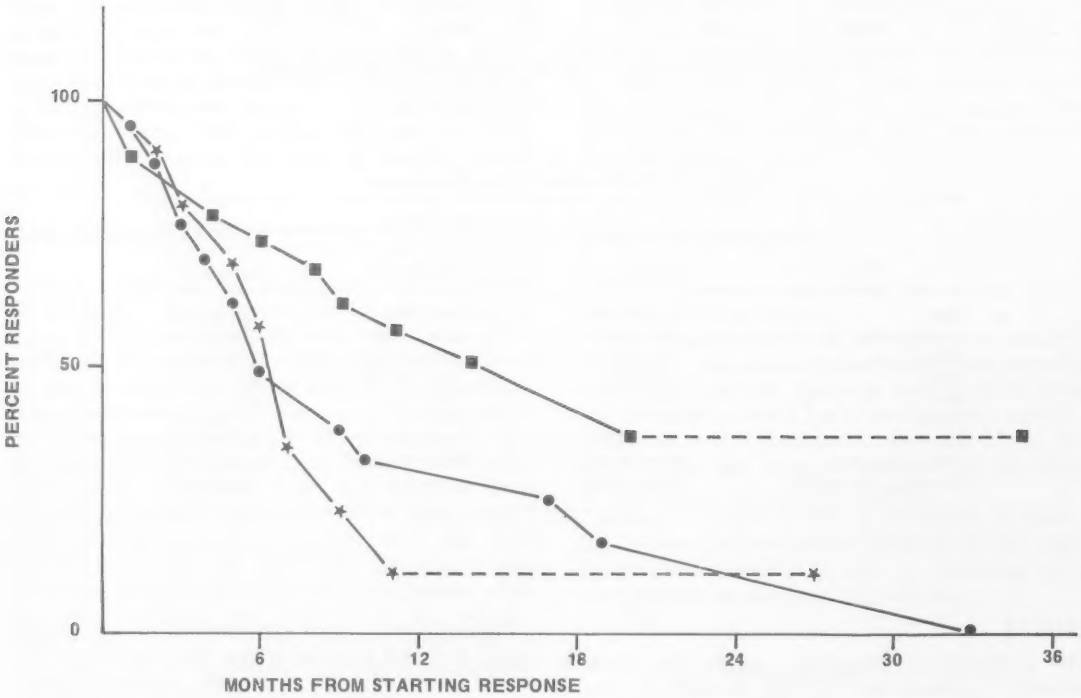
Table 3 presents the response in relation to the different sites of metastatic deposits. As has been shown in other studies (2), a higher number of responses was observed in soft tissue disease as compared to visceral disease. There were no statistical differences in the response rates among the three regimens.

Figures 2 and 3 show the survival curves for the responders and nonresponders. The median survival was improved in all responders without a significant difference among the three regimens. In nonresponders the median survival was < 6 months.

In all three regimens no statistical difference was detected in terms of response rate and survival between men and women. The involvement of the central nervous system (CNS) was analyzed at different times in relation to therapy. The median survival from the onset of brain metastases was < 2 months (range, 0.1-9 mos). The most important finding was that the incidence of CNS metastases (during treatment or after its discontinuation) was neither lowered nor delayed in the groups of patients receiving either regimen A or C which included BCNU.

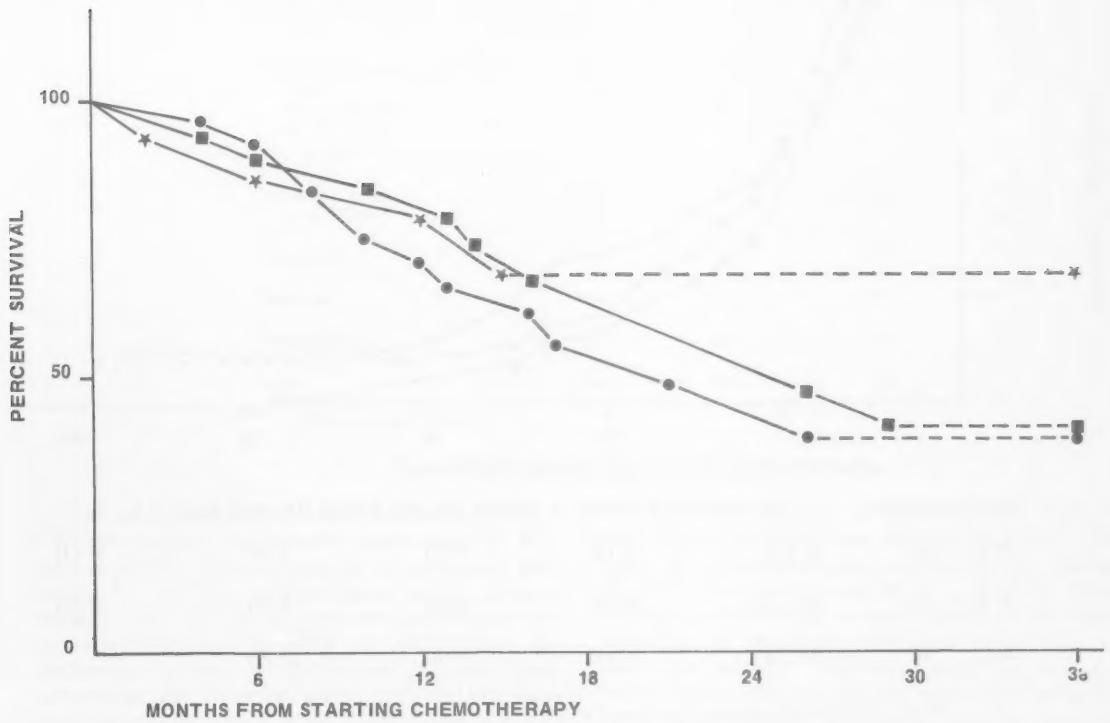
Toxic manifestations are summarized in table 4. Hematosuppression and especially marked leukopenia (white blood cell [wbc] count = 1999-1000 cells/mm^3) were more pronounced in regimen C and these findings can be attributed to the presence of

actinomycin D in the combination. This also explains the higher incidence of stomatitis and hair loss in regimen C compared with regimens A and B. Neurotoxicity (usually paresthesias and loss of deep tendon reflexes secondary to the administration of vincristine) only rarely led to a reduction in the drug dose. No drug-related deaths were reported.



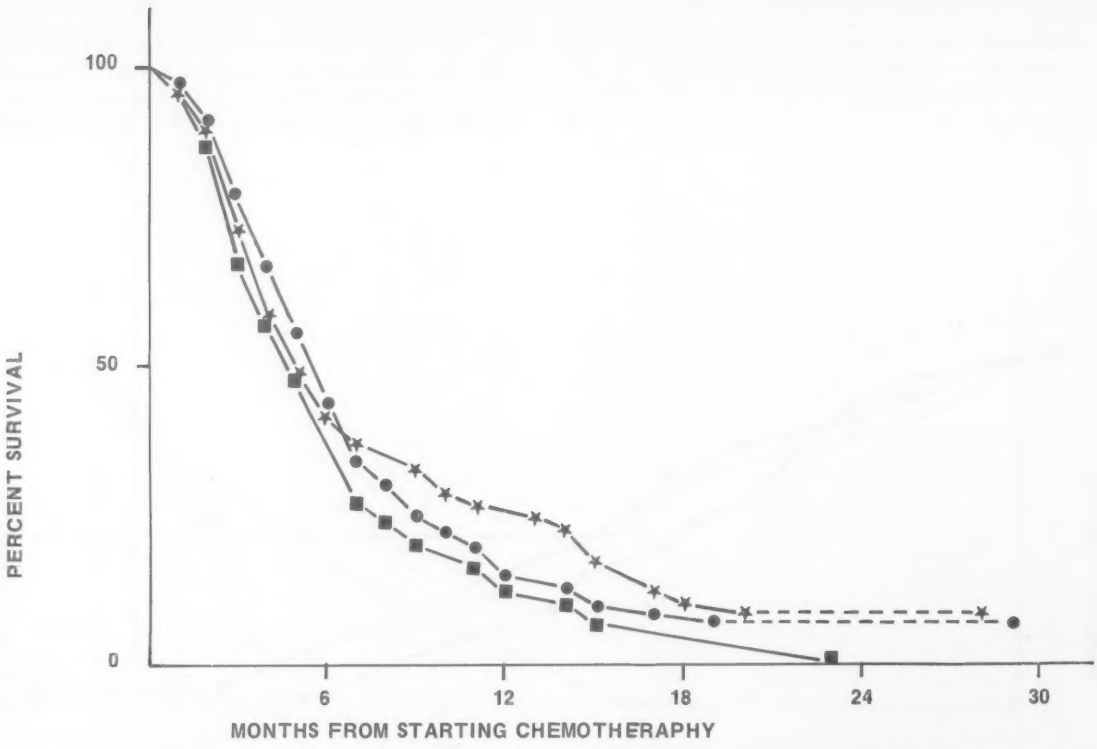
TREATMENTS		PATIENTS AT RISK * (WITH DRAWN FREE OF DISEASE **)					
● A	27	11*(3)**	5 (3)	3 (1)	2 (0)	1 (1)	0 (0)
✱ B	17	6 (8)	1 (0)	1 (0)	1 (0)	0 (1)	
■ C	19	13 (1)	10 (1)	8 (1)	3 (3)	1 (2)	0 (1)

FIGURE 1.—Actuarial analysis of duration of CRs plus PRs.



TREATMENTS		PATIENTS AT RISK * (WITH DRAWN ALIVE **)					
● A	27	24*(2)**	17 (2)	8 (5)	5 (2)	4 (0)	2 (2)
✱ B	17	13 (3)	9 (2)	4 (4)	1 (4)	1 (0)	1 (0)
■ C	19	17 (0)	16 (1)	12 (0)	11 (1)	5 (2)	1 (4)

FIGURE 2.—Actuarial analysis of survival of CRs plus PRs.



TREATMENTS		PATIENTS AT RISK * (WITH DRAWN FREE OF DISEASE **)				
● A	102	39 (12)	12 (2)	5 (2)	1 (3)	0 (1)
✱ B	67	24 (7)	12 (4)	5 (1)	3 (0)	0 (3)
■ C	42	14 (6)	4 (1)	1 (0)	0 (0)	

FIGURE 3.—Actuarial analysis of survival of patients with objective improvement or no response.

DISCUSSION

The results of our controlled study confirm once more that advanced malignant melanoma remains a difficult disease to control with chemotherapy. In terms of response rate, 27 of 129 (20.9%) adequately treated patients who were given the combination of DTIC, vincristine, and BCNU had either CR or PR. The results are in the range of those already reported in a similar series of 106 patients treated by Einhorn et al (3).

The two new combinations which included actinomycin D and hydroxyurea respectively yielded a similar response rate. In fact, although the percentage of CRs was significantly less in the regimen where BCNU was replaced by hydroxyurea, the median duration of CR plus PR in this regimen was comparable to the other two regimens. Also, when the response was related to the pattern of metastatic extent, no statistical difference was detected among the three triple combinations either at the level of soft tissue involvement or in visceral melanoma.

In patients with advanced melanoma the analysis of survival also confirms that responders live longer than nonresponders. Unfortunately, this study also shows that CRs were limited to a small fraction of patients with cutaneous and/or nodal involvement as well as to a small percentage of those with only pulmonary metastases.

Our findings provide virtually conclusive evidence that BCNU failed both to produce a response

TABLE 4.—Percent toxic manifestations

Side effects	Regimen A	B	C
Hematosuppression	38.7	22.9	56.4
Leukopenia (wbc cells/ mm³)	24.7	18.7	24.2
4000-2000	1	2	14.5
1999-1000	1	—	1.6
<1000			
Thrombocytopenia (platelets/ mm³)	10.7	16.6	19.3
150,000-100,000	5.3	2	—
99,999-50,000	1	2	1.6
< 50,000			
Anemia (hemoglobin <3 g/100 ml)*	10.7	2	22.5
Stomatitis	4.3	2.1	16.1
Hair loss	19.3	2.1	48.3
Neurotoxicity	34.4	14.5	1.6
None	12.9	10.4	4.8

*Below initial values.

in CNS metastases and to delay the development of CNS involvement. As recently emphasized by Einhorn et al (3), CNS involvement in malignant melanoma is a serious problem which at the present moment can only be temporarily ameliorated by corticosteroids and possibly by whole-brain radiotherapy. In fact, our data show that once brain metastases are detected either through physical examinations or brain scans, the median survival is short.

With the dose schedules used, toxic effects were found to be moderate, with the exception of stomatitis occurring in the regimen using actinomycin D. Considering that the response rate as well as the median duration of response and survival time obtained with this treatment were not statistically different from those achieved with DTIC, vincristine, and BCNU, there is no sufficient reason to prefer the three-drug regimen including DTIC, BCNU, and actinomycin D over the above-mentioned combination. In conclusion, our controlled study fails to show the superiority of any one of the three-drug regimens tested. Furthermore, the percentage of CRs plus PRs as well as the response rates in visceral disease obtained with each combination were not superior to those obtained by several investigators who used DTIC alone (2).

Finally, the addition of BCNU to the combination neither decreased nor delayed the clinical incidence of brain metastases, supporting the findings of Costanza et al (4) and Einhorn et al (3). These findings underline the current status of the chemotherapy of metastatic melanoma which has not been substantially improved during the past few years. Since some melanomas have a potential for early dissemination and many patients have regional node metastases at the time of the radical surgery of the primary tumor, a combined treatment approach with surgery, chemotherapy (single agent or combination), and/or immunotherapy is the field where clinical efforts should be concentrated. In fact, there is some preliminary evidence that bacillus Calmette-Guerin and DTIC or the combination of both can increase the response rates in patients with disseminated disease (5,6) and prolong the disease-free survival time in patients without clinically demonstrable melanoma (7).

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Randomized Clinical Comparison of Daunorubicin (NSC-82151) Alone With a Combination of Daunorubicin, Cytosine Arabinoside (NSC-63878), 6-Thioguanine (NSC-752), and Pyrimethamine (NSC-3061) for the Treatment of Acute Nonlymphocytic Leukemia[1,2]

Peter H. Wiernik,* Stephen C. Schimpff, Charles A. Schiffer, J. Leonard Lichtenfeld, Joseph Aisner, Michael J. O'Connell, and Clarence Fortner[3]

SUMMARY

Sixty-six newly diagnosed patients with acute nonlymphocytic leukemia received either daunorubicin alone or a combination of daunorubicin, cytosine arabinoside, 6-thioguanine, and pyrimethamine for remission-induction therapy. The two treatment groups were comparable with respect to the two major prognostic factors in this disease, which were age and presence or absence of infection on admission. The two therapies produced similar results with respect to CR rate and median survival results. Single-agent therapy was associated with less frequent utilization of hospital inpatient facilities and fewer platelet transfusions. The four-drug combination did not decrease the incidence of meningeal leukemia. Patients who achieved CR were treated with two half-dose consolidation courses of the successful remission-induction regimen. Subsequently, all patients received cyclophosphamide and guanazole monthly for maintenance therapy. Median durations of remission for both induction-treatment groups were similar (6.8 and 5.6 mos). The therapeutic results with the single agent in this study were not inferior to those obtained with the drug combination tested, as well as most other previously reported combinations of antileukemic drugs.

[Cancer Treat Rep 60:41-53, 1976]

Improved management of adults with acute nonlymphocytic leukemia (ANLL) with intensive drug therapy and maximal supportive care has resulted in a definite increase in survival for patients treated by specialized disease-oriented clinical teams (1). Since only patients who achieve CR after such therapy benefit significantly from treatment (1), it will be necessary to develop new drugs, or new combinations of currently available drugs, that will result in CR rates superior to those currently achievable.

To date, CR maintenance in patients with ANLL has been difficult (2). If a major impact is to be made on the survival of patients who achieve remission, more effective means of prolonging the clinically disease-free state must be devised.

Meningeal leukemia is recognized as a distressing syndrome in its own right and a harbinger of the incurable state in acute lymphocytic leukemia (ALL) (3). Effective prophylactic therapy for meningeal leukemia has been developed for children with ALL and its use has resulted in a decreased incidence of systemic relapse in that disease and, therefore, improved disease-free survival (4). Meningeal leukemia is emerging as a significant clinical problem in adults with ANLL who now survive longer than before (5). While the influence of meningeal leukemia on systemic relapse in patients with ANLL is less clear than in patients with ALL, it is reasonable to suspect that they are related in both diseases.

[1]Received June 18, 1975; revised Nov 18, 1975; accepted Nov 19, 1975.

[2]Daunorubicin: CAS reg. No. 23541-50-6.

Cytosine arabinoside: CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride.

6-Thioguanine: CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-.

Pyrimethamine: CAS reg. No. 58-14-0.

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This study was begun in July 1971 to determine if a combination of the three most effective agents for the induction of CR in patients with ANLL (daunorubicin, cytosine arabinoside, and 6-thioguanine) might result in a CR rate superior to that obtained with daunorubicin alone, the single most effective agent for this disease (6). The study was further designed to test the efficacy of intermittent cyclophosphamide and guanazole (7)[4] as a remission-maintenance regimen. In addition, pyrimethamine, an orally administered weak dihydrofolate reductase inhibitor (8), was tested as an agent for the prophylaxis of meningeal leukemia. Its usefulness in this regard was suggested by its alleged activity in overt meningeal leukemia in patients with ANLL (9,10). The results reported in this paper are as of September 1, 1975.

MATERIALS AND METHODS

Patients

Sixty-six previously untreated, unselected, and consecutive patients were randomly allocated to one of the two induction-treatment regimens. All patients were considered evaluable at the end of the study (table 1). The 33 patients (22 men) in the single-agent induction group had a median age of 51 years (18% > 70 yrs) and a mean white blood cell (wbc) count of 32,800 cells/mm^3. The 33 patients (19 men) in the combination therapy induction group had a median age of 53 years (9% > 70 yrs). The mean platelet count for both groups was 50,000 cells/mm^3 and each treatment group included 12 patients who had significant infections. All patients had at least 25% blast cells in bone marrow aspirates and progressive granulocytopenia and thrombocytopenia before therapy was started.

Supportive Care

Some form of protection against the acquisition of infectious organisms during induction therapy was used in all but two patients in the single-agent group who did not have infection and in all but four uninfected patients in the combination group. Eight patients given single-agent therapy were treated in laminar air flow rooms and were given oral nonabsorbable antibiotics (gentamicin, vancomycin, and mycostatin) prophylactically (11) as were seven patients in the combination-therapy group. In addition, seven patients in the single-agent group and six in the combination-therapy group were treated with oral nonabsorbable antibiotics without isolation. Patients treated in the laminar air flow rooms and/or with oral antibiotics received that supportive therapy until CR was achieved or death occurred.

Random donor or HL-A-compatible platelet transfusions were given to all bleeding patients and prophylactically to patients whenever the platelet count was < 20,000 cells/mm^3 (12). HL-A-compatible granulocyte transfusions prepared by filtration leukopheresis (13) were given to three infected granulocytopenic patients in the single-agent therapy group and to six in the combination therapy group during induction therapy. Two patients given single-agent therapy and three receiving combination therapy required heparinizatio.. during induction therapy for bleeding associated with hypofibrinogenemia and elevated fibrin split products (14).

TABLE 1.—Patient data—pretreatment

	Treatment group	
	Daunorubicin alone	Combination therapy*
No. of patients entered	33	33
No. of patients evaluable	33	33
Median age (yrs)	51	53
Mean wbc (cells × 10^3/mm^3)	32.8	31.3
Mean blood blasts (%)	37	48
Mean absolute blast count (cells × 10^3/mm^3)	19	24
Mean platelet count (cells × 10^3/mm^3)	50	50

*Daunorubicin, pyrimethamine, 6-thioguanine, and cytosine arabinoside.

Morphology

The cell type in the bone marrow was assigned to a morphologic subgroup of ANLL according to standard criteria (15) with the following results: 16 patients of the single-agent group and 14 of the combination group had acute myelocytic leukemia (AML); 12 of the single-agent group and 11 of the combination group had acute myelomonocytic leukemia (AMML); three of the single-agent group and six of the combination group had acute monocytic leukemia; one of the single-agent group and two of the combination group had acute promyelocytic leukemia; and one of the single-agent group had Di Guglielmo's disease.

[4]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Guanazole: NSC-1895; CAS reg. No. 503-88-8; 1,2,4-triazolidine, 3,5-diamino-.

Chemotherapy

Chemotherapy consisted of three phases of treatment in both study groups: a remission-induction phase followed by a remission-consolidation phase and a cytoreductive-maintenance phase (fig 1).

Induction phase.—Patients allocated to the single-agent group received three consecutive daily doses of daunorubicin (60 mg/m^2) given by rapid iv injection. Patients in the combination-therapy group received a 5-day course of therapy. On Day 1 daunorubicin (75 mg/m^2) was administered by rapid iv infusion, and on Days 1-5 cytosine arabinoside and 6-thioguanine were each given at a dose of 75 mg/m^2 every 12 hours, the former by rapid iv infusion and the latter orally. In addition, pyrimethamine (1 mg/kg/day) was given orally on Days 1-5. Frequent bone marrow examinations were performed after induction therapy (usually every 3-5 days). If, after a hypocellular marrow was achieved, the marrow became repopulated with blast forms, another marrow examination was performed within 3-5 days to ensure the malignant nature of the repopulating cells (16). Patients were taken off the study and considered induction-therapy failures if they did not achieve CR with a maximum of three courses of therapy or if they died during the therapy.

Consolidation phase.—Patients who achieved CR were treated with two half-dose courses of the successful remission-induction regimen after the bone marrow and peripheral blood counts recovered from induction therapy. Prior to consolidation therapy all patients had a normocellular marrow with a normal differential count, a platelet count of at least 150,000 cells/mm^3, and a circulating granulocyte count of at least 1500 cells/mm^3. Time was allotted between consolidation courses for similar hematologic recovery, usually 2 weeks.

Cytoreductive-maintenance phase.—After hematologic recovery from the consolidation-treatment phase, all patients in both treatment groups who were still in CR received three monthly courses of cyclophosphamide (a non-cell-cycle-specific agent [1000 mg/m^2]) given as a rapid iv injection and followed in 18 hours by a 24-hour iv infusion of guanazole (an S-phase active agent [25 g/m^2]). After hematologic recovery from the last course of cyclophosphamide and guanazole, the patients in the combination induction-therapy group received pyrimethamine (2 mg/kg) as a single oral dose given weekly until systemic and/or central nervous system (CNS) relapse occurred.

Criteria for Response

CR was diagnosed according to the criteria of the Acute Leukemia Group B (ALGB) (17). The patients considered to be in CR were asymptomatic, had no physical findings suggestive of leukemia, and had nondiagnostic bone marrow examinations and normal peripheral blood counts. Patients with PR were also asymptomatic and had normal peripheral blood counts and improved bone marrow examinations with < 25% leukemic cells.

Tests of Delayed Cutaneous Hypersensitivity

Thirteen patients in the combination-therapy group and 11 in the single-agent group had one to seven tests of cutaneous delayed hypersensitivity reaction prior to antileukemic therapy.[5] The results were correlated with remission and survival data.

RESULTS

Induction therapy.—Each of the 66 patients entered in this study was considered evaluable, in-

[5]Coccidioidin, dermatophytin, histoplasmin, mumps, tuberculin (purified protein derivative) antigens as well as streptokinase-streptodornase were applied as previously described (18). In addition, sensitizing and challenging doses of dinitrochlorobenzene were applied to some patients as previously described (18). All patients except one received at least four tests.

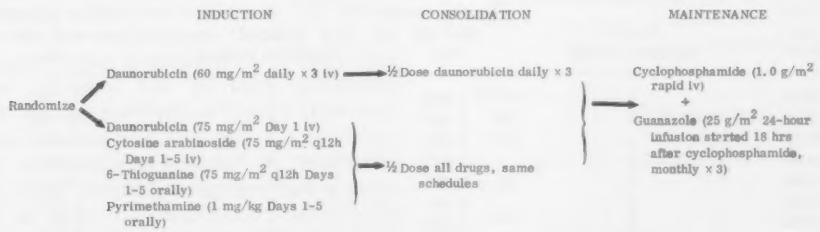
INDUCTION
CONSOLIDATION
MAINTENANCE
Randomize
Daunorubicin (60 mg/m2 daily x 3 iv)
½ Dose daunorubicin daily x 3
Daunorubicin (75 mg/m2 Day 1 iv)
Cytosine arabinoside (75 mg/m2 q12h Days 1-5 iv)
6-Thioguanine (75 mg/m2 q12h Days 1-5 orally)
Pyrimethamine (1 mg/kg Days 1-5 orally)
½ Dose all drugs, same schedules
Cyclophosphamide (1.0 g/m2 rapid iv)
+
Guanazole (25 g/m2 24-hour infusion started 18 hrs after cyclophosphamide, monthly x 3)

FIGURE 1.—Schematic chart of chemotherapy employed in the study.

cluding two patients in the single-agent induction group who died within 10 days of admission to the hospital; one of these patients died after receiving only two of three planned doses of daunorubicin for the first induction course. The results of the induction therapy are presented in table 2. The CR plus PR rate was 52% for the single-agent group and 58% for the combination-therapy group, and the CR rates for the two groups were also similar (49% vs 46% respectively). Four patients survived an unsuccessful trial of daunorubicin alone, one of whom subsequently achieved CR with other therapy. There were no survivors among the combination-therapy failures. The response rates varied with age but CRs were obtained in patients 15-76 years of age. Table 3 indicates the CRs according to age and treatment regimen.

The infectious-disease status of patients prior to therapy influenced the response rates in both induction-treatment groups. Thirty-six percent of the patients in each treatment group entered the hospital with significant infections and a minority of them achieved CR. On the other hand, 57% of the patients treated with either combination chemotherapy or single-agent induction therapy who had no significant infection prior to the start of induction therapy achieved CR. The mean number of induction-therapy courses necessary to achieve CR in this study was similar for both groups (1.6 courses for daunorubicin alone and 1.8 courses for combination therapy). One patient in each group achieved CR after the third course of therapy. Therapy was arbitrarily stopped in this study after failure of three induction courses (table 2).

TABLE 2.—Induction-therapy results

	Treatment group	
	Daunorubicin alone	Combination therapy*
No. of patients treated	33	33
CR	16 (49%)	15 (46%)
PR	1 (3%)	4 (12%)
Mean No. of treatment courses to CR	1.6	1.8
No. of complete responders achieving CR with 1 course	7/16 (44%)	4/15 (27%)
No. of complete responders achieving CR in < 30 days	10/16 (63%)	4/15 (27%)
No. of patients who died during therapy	12	14
No. of patients surviving unsuccessful trials	4	0

*See table 1 for definition.

TABLE 3.—Effect of age on induction-therapy results

	Treatment group			
	Daunorubicin alone		Combination therapy*	
Patient age (yrs)	No. of patients treated	No. of patients with CR (%)	No. of patients treated	No. of patients with CR (%)
10-19	3	1 (33)	1	1 (100)
20-29	6	3 (50)	3	3 (100)
30-39	4	3 (75)	4	2 (50)
40-49	3	1 (33)	6	3 (50)
50-59	8	6 (75)	10	4 (40)
60-69	3	0 (0)	6	2 (33)
70-79	6	2 (33)	3	0 (0)
Total	33	16 (49)	33	15 (46)

*See table 1 for definition.

Consolidation therapy.—Patients in the single-agent group spent a mean of 4.6 days (range, 0-18 days) in the hospital receiving both courses of consolidation therapy. All except two patients with CR received both planned consolidation courses. One patient relapsed after one course; one patient refused further therapy and his CR duration was only 2.6 months. The mean hospitalization time during consolidation therapy for the combination-therapy patients was 9.5 days (range, 0-38 days). One patient in this group also only received one consolidation course because of relapse prior to the second course.

Cytoreductive-maintenance therapy.—Eleven of the 16 single-agent therapy patients who achieved CR received cytoreduction therapy. Two patients refused the therapy and three relapsed before the first course could be given. One patient relapsed after the first course at the time the second course was due. Two patients relapsed after the second course and eight completed all three courses. Two of the 15 patients achieving CR in the combination-therapy group relapsed before cytoreduction therapy was begun, one relapsed after the first course, and one relapsed after the second course. Thus, 11 patients in this group received all three cytoreduction courses. In both treatment groups approximately 6.5 days (mean) were required in the hospital to receive from one to three cytoreduction courses.

Remission Duration and Survival

The median duration of CR was 6.8 months (range, 2.0-15.8 mos) and 5.6 months (range, 1.5-10.1 mos) for the single-agent and combination-therapy groups respectively. Five patients (one in the single-agent therapy group and four in the combination-therapy group) achieved PR. One PR lasted 9.7 months in the combination-therapy group while the three other PRs in that group were of 1 month's duration or less. A 2-month PR was obtained in the one single-agent induction therapy patient achieving that status.

The median durations of survival for all treated patients in the two induction groups in this study are 11.6 months for single-agent therapy and 5.2 months for combination therapy ($P = < 0.05$). The patients who achieved CR in both treatment groups had a median survival > 1 year (13.8 mos for daunorubicin alone and 12.6 mos for combination therapy) while patients who did not respond to therapy with either induction regimen had a median survival of only 1.3 months. Patients who achieved PR had an intermediate survival: a 5.0-month median survival for the four patients treated with combination therapy and a 7.3-month survival for the one patient in the single-agent group.

Fourteen single-agent therapy patients have survived for > 1 year and 12 of those patients achieved CR. Thus, 75% of the patients achieving CR with this induction regimen have survived 1 year or more. On the other hand, only 42% of *all* patients treated with daunorubicin alone survived that long.

Nine combination-therapy patients survived at least 1 year with eight achieving a CR and one achieving a PR. Therefore, only 53% of those patients who achieved CR survived at least 1 year as did 27% of all the patients treated with combination therapy. These data are inferior to the single-agent therapy data. Six patients have survived at least 18 months in each induction group; five in the single-agent group were complete responders as were all six in the combination-therapy group.

In the group given daunorubicin alone, the patients with wbc counts > 75,000 cells/mm^3 who achieved CR had a shorter median duration of remission than the complete responders who had wbc counts < 20,000 cells/mm^3 (3.2 vs 7.1 mos, $P = 0.03$), although survival of complete responders was similar without regard to the initial wbc counts (12.3 vs 13.8 mos median). The same trend was noted in the combination-therapy patients who achieved CR. The median duration of remission was longer in patients who had a wbc count < 20,000 cells/mm^3 compared to those who had a wbc count > 75,000 cells/mm^3 (7.2 vs 5.4 mos, $P = > 0.05$), but there was no difference in the median survival between the two wbc groups for complete responders (18.1 vs 18.6 mos).

Significant differences in survival were noted in both treatment groups with respect to patient age. Survival was superior for patients < 60 years of age in both treatment groups compared to that of older patients ($P = < 0.05$ for combination therapy and $P = < 0.01$ for single-agent therapy) (table 4).

TABLE 4.—Age-related survival for all treated patients

Patient age (yrs)	Median survival (mos) for—	
	Daunorubicin alone	Combination therapy*
<60	11.7	8.2
>60	1.1	2.5

*See table 1 for definition.

Supportive Care

Patients in both treatment groups spent a similar number of days in the hospital prior to the start of specific antileukemic therapy (mean No. of days, 3.1). Hospitalization was shorter for single-agent therapy patients who achieved CR (median, 28.5 days) than for those who completely responded to combination therapy (median, 40.0 days). Some patients not responding to therapy spent considerably more time in the hospital. Nonresponders in the single-agent group spent a median of 21 days in the hospital and combination-therapy nonresponders spent a median of 35 days. The time in the hospital represented a significant portion of post-diagnosis survival for nonresponders in both treatment groups. Nonresponders in the combination-therapy group were hospitalized a mean of 73% of the post-diagnosis survival time (range, 24%-97%) and nonresponders in the single-agent therapy group a mean of 56% (range, 20%-100%) (table 5).

In the single-agent group, patients had wbc counts < 1500 cells/mm^3 for a mean of 29 days (range, 12-107 days) during induction therapy and in the combination-therapy group the wbc count was < 1500 cells/mm^3 for a mean of 24 days (range, 0-46 days). All patients in the single-agent therapy group required systemic antibiotic therapy for suspected or proved infection during induction therapy for a mean of 16 days (range, 1-84 days). Patients in the combination-therapy group received systemic antibiotics for a mean of 17 days (range, 0-41 days). However, two of the patients in this group who subsequently achieved CR did not require such therapy. No patient in either group required antibiotics during consolidation or cytoreduction therapy.

Platelet transfusions were required for an average of 12 days for patients treated with combination therapy and for an average of 10 days for single-agent therapy patients during induction therapy. Patients who achieved CR required platelet transfusions on an average of 9 and 7 days during induction with combination and single-agent therapy respectively. The combination group received an average of 136 platelet units/patient and the single-agent group required 93 units/patient. For complete responders, the figures are 95 and 67 units for the respective therapy groups. Thus, the single-agent regimen was less costly from a platelet transfusion standpoint. Red blood cells (rbc) were transfused on an average of every 6 days to patients in each treatment group and complete responders required rbc transfusions on an average of 4-5 days. Patients on both treatment regimens received an average of 13-14 units of rbcs during induction therapy; the patients completely responding to induction therapy required fewer rbc units (an average of 10 units in both therapy groups) (table 6).

Skin Test Results

Ten of the 13 patients in the combination-therapy group tested had at least one positive test and seven of the ten achieved a CR to induction therapy. One of two partial responders who were tested had a positive response to one test and both nonresponders who were tested reacted positively to at least one skin test. In the single-agent induction therapy group, five of 11 skin-tested patients

TABLE 5.—Hospitalization time required

Time in hospital—	Treatment group	
	Daunorubicin alone	Combination therapy*
Prior to treatment (median, days)	3.1	3.2
For induction therapy in patients achieving CR (median, days)	28.5	40.0
For induction therapy in nonresponding patients (median, days)	21.0	35.0
Mean % of survival time spent in hospital (nonresponding patients)	56	73

*See table 1 for definition.

TABLE 6.—Mean transfusion requirements per patient

	Treatment group	
	Daunorubicin alone	Combination therapy*
No. of days platelets transfused		
All patients	10	12
CR patients	7	9
Total No. of platelet units transfused		
All patients	93	136
CR patients	67	95
No. of days rbcs transfused		
All patients	6	6
CR patients	5	4
Total No. of rbc units transfused		
All patients	13	14
CR patients	10	10

*See table 1 for definition.

achieved CR and four of those five had at least one positive reaction to intradermal antigen. The one partial responder who was skin tested did not react to intradermal antigen and three of five tested nonresponders had a positive reaction to at least one skin test. Thus, delayed cutaneous hypersensitivity status prior to therapy did not appear to influence the results of therapy in this study.

Meningeal Leukemia

Two patients in each induction-therapy group had diagnostic cerebrospinal fluid (CSF) findings of CNS leukemia prior to induction therapy. All four patients responded to appropriate therapeutic measures. Three of the four achieved CR and survived > 1 year and none of the four had further episodes of CNS leukemia.

Four combination-therapy patients developed diagnostic findings of CNS leukemia from 95 to 222 days after the diagnosis of acute leukemia, and in three patients the onset of CNS leukemia was asymptomatic and the diagnosis was made only through scheduled monthly lumbar punctures. The fourth patient had lower limb weakness and paresthesias. All four patients responded to appropriate therapy and survived from 72 to 312 days after the development of CNS leukemia without recurrent episodes. Three of the four patients developed CNS leukemia during CR and the fourth patient did so during a PR.

Only three single-agent therapy patients developed meningeal leukemia after induction therapy was started, but two of the three had significant headaches at the time CNS leukemia was diagnosed. CNS leukemia was diagnosed in these three patients from 74 to 98 days after acute leukemia was diagnosed, and two of the three patients were in CR at the time. The three patients responded to appropriate therapy and survived from 108 to 390 days after CNS leukemia was diagnosed. However, one patient had recurrent episodes requiring repeated therapy. In two of the three patients hematologic relapse occurred within 1 month of the onset of CNS leukemia. In the third patient, a CR lasting 8.8 months began shortly after the resolution of CNS leukemia.

The incidence of meningeal leukemia is similar in these two groups of patients, a fact which argues against the value of pyrimethamine in the prophylaxis of this complication.

Pyrimethamine was measured in serum and CSF in five patients treated with the combination therapy arm of this study by Dr. Dwight R. Stickney, Burroughs-Wellcome Laboratories, Research Triangle Park, North Carolina (19). Concentrations of 0.2-0.4 μg/ml were found in the CSF at the end of the initial 5-day combination induction-therapy course and serum levels as high as 2.04 μg/ml were achieved at that time. The drug could be detected in the CSF as long as 3 weeks after a 5-day course in some patients. Pyrimethamine given orally does, therefore, enter into the CSF in measurable amounts and remains there for prolonged periods of time. A more detailed analysis of this data will be reported separately.

Marrow Morphology

The influence of marrow morphology on the effect of therapy in this study is examined in table 7. The majority of patients in both induction-therapy groups were classified as AML or AMML according to previously published criteria (15). In both treatment groups the patients with AMML had a longer median survival and a higher remission rate than those patients with AML. In addition, complete responders with AMML in both treatment groups had a greater median survival than did AML patients who completely responded to the same regimen. This effect was more evident in patients treated with combination therapy than in those treated with daunorubicin alone. Too few patients with other subtypes of ANLL were entered into this study to be evaluated in this way.

The marrows of 30 patients in this study were blindly classified as to the degree of leukemic cell

TABLE 7.—Influence of marrow morphology on response and survival

	Disease	
	AMML	AML
Combination therapy*		
No. of patients treated	11	14
Median survival (mos)	13.4	1.6†
CR (%)	70	21†
CR median duration (mos)	5.6	3.7
CR median survival (mos)	20.3	10.6†
Daunorubicin alone		
No. of patients treated	12	16
Median survival (mos)	12.6	6.0†
CR (%)	58	44
CR median duration (mos)	6.7	7.4
CR median survival (mos)	14.2	12.8

*See table 1 for definition.
†$P = < 0.05$.

TABLE 8.—Effect of marrow differentiation on therapy response

Brincker classification	Treatment group: Daunorubicin alone (18 patients)	Treatment group: Combination therapy* (12 patients)
A (least differentiation)	2/3† (67%)	1/6† (17%)
B	4/8† (50%)	3/6† (50%)
C (greatest differentiation)	3/7† (43%)	—

*See table 1 for definition. See text for details.
†No. of patients with CR/No. of patients treated.

differentiation by Dr. Hans Brincker, Odense, Denmark. According to his system (20) "A" represents the least and "C" the greatest differentiation of the involved cell line(s) in a leukemic marrow. Although only small numbers were represented in each category in this study, the system did not appear to be of value in retrospectively identifying those patients most likely to respond to chemotherapy (table 8).

Toxicity

All patients had marked and most had total capital alopecia which was usually maximal after the first course of daunorubicin alone and after the second course of combination therapy. Slightly more than half of the single-agent therapy patients had nausea and vomiting within several hours of drug administration as did 75% of the patients on combination therapy. Drug-related stomatitis occurred in five patients treated with daunorubicin alone and in three receiving combination therapy. Temperature elevations of 1°F-3°F related to drug administration occurred in eight patients who received single-agent therapy and in ten who received the combination treatment. Hematologic toxicity was pronounced in virtually all patients and this was considered a necessary result of potentially successful therapy. In all but one patient in each induction-treatment group marked marrow hypocellularity and pancytopenia occurred. Measures to minimize the potentially lethal results from such hematologic depression have been previously discussed.

Consolidation therapy was well tolerated and toxicity (including hematologic) was relatively minor. Maintenance cytoreduction therapy produced nausea and vomiting in every patient and moderate to severe hematologic depression that was usually resolved within 10 days of therapy.

TABLE 9.—Relation of daunorubicin cardiotoxicity to total dose

Treatment group and dose (mg/m^2)	No. of patients treated	No. with cardiotoxicity
Daunorubicin alone		
≤ 540	18	0
540-840	9	2 (22%)
> 840	6	2 (33%)
Total	33	4
Combination therapy*		
≤ 540	25	0
540-840	5	0
> 840	3	0
Total	33	

*See table 1 for definition.

Capital alopecia and occasional drug-related rashes (four patients) also occurred during this phase of therapy.

Daunorubicin cardiac toxicity has been observed in four patients in this study. All four had originally received daunorubicin alone as induction therapy. Of the four patients with cardiotoxicity, two had a CR and one had a PR. These patients ranged from 10 to 56 years of age and had received daunorubicin at an average dose of 889 mg/m^2 (range, 720-1065) during their lifetimes. It is interesting to note that while four of 14 single-agent therapy patients who received > 540 mg/m^2 of daunorubicin developed cardiotoxicity, none of the eight combination-therapy patients did ($P = < 0.1$, > 0.05). These data suggest that time, as well as dose factors, may be important in the development of daunorubicin cardiotoxicity (table 9).

Postrelapse Course

Seven of the 15 combination-therapy complete responders (47%) had two to six CRs. Five patients achieved a second CR with the same combination of agents and two did so with 5-azacytidine (21).[6] The duration of a second CR ranged from 16% to 90%+ of the duration of the first remission, with a median of 51%. The median survival time after the first relapse for combination-therapy complete responders was 5.3 months with five patients surviving > 1 year after relapse. All five had multiple CRs.

[6]5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-.

Seven of the 16 single-agent therapy complete responders (44%) had two to three CRs. Five patients in this group achieved a second CR with the same induction therapy that was initially successful and two had a second CR induced with the combination therapy used in the other arm of this study. The duration of a second CR in single-agent therapy patients ranged from 9% to 358% of the duration of the first remission, with a median of 40%. The median survival time after the first relapse for single-agent therapy responders was 6.3 months with four multiple-remission patients surviving > 1 year after relapse.

COMMENTS

The 66 patients in this study were randomly allocated to one of two therapeutic regimens for remission induction of ANLL. The groups were essentially identical with respect to median age, mean peripheral blood counts prior to therapy, and number of patients with significant infections upon admission to the hospital. The purpose of the induction trial was to determine if the reasonable results reported by us (6) and others (22) with daunorubicin alone would be improved upon by combining that agent with two other highly active agents for the treatment of ANLL (6-thioguanine and cytosine arabinoside) (23). In addition, pyrimethamine, an oral antifol with reported activity against meningeal leukemia in ANLL (9), was added to the three-drug combination to determine its role, if any, in the prophylaxis of meningeal leukemia in patients with ANLL. The CR rates obtained with daunorubicin alone and with the combination treatment were virtually identical (49% vs 46% respectively). In addition, one third of the patients failing daunorubicin therapy survived that therapy and went on to other induction therapy regimens, whereas none of the combination-therapy failures survived that induction treatment.

As noted with other induction trials in ANLL (24), the mean wbc count in both therapeutic groups in this study was higher in patients who achieved a CR than in those who did not respond to therapy. The patients who achieved a PR had intermediate initial wbc counts. This relationship of wbc counts to response rates has not been previously observed with cytosine arabinoside (24) or daunorubicin (25) alone.

Daunorubicin was superior to the combination therapy in the time required for induction of a CR. Almost two thirds of the patients achieving a CR with daunorubicin alone did so in < 30 days compared to only approximately one fourth of the complete responders to the combination therapy.

The median CR duration for patients responding to daunorubicin therapy was 6.8 months compared to 5.6 months for those obtaining CR with combination therapy. These figures are *actual* rather than actuarial projections. The 6.8-month median duration of remission obtained with daunorubicin alone in this study is superior to the 2.3-month median CR duration obtained with daunorubicin in a previous study at this institution (6) in which no consolidation or maintenance therapy was given. These data suggest that consolidation therapy with daunorubicin after CR is achieved and/or cytoreductive-maintenance therapy as administered in this study may prolong remission duration. While the median durations of remission obtained in this study do not compare favorably with some other studies, it must be remembered that monthly bone marrow examinations were done in this study, and remission was considered terminated when > 5% blast cells appeared in a regularly scheduled bone marrow examination irrespective of peripheral blood findings. Sometimes this determination was made retrospectively by reexamining serial marrow specimens after a clinically significant relapse had clearly occurred. In such cases, remission duration was adjusted downward for the purposes of this evaluation. Many of the patients who relapsed did not require therapy for several months or more after the relapse was officially documented. It is the policy of some investigators to increase maintenance therapy at a time that we would have judged the patient to be in relapse, and therefore to artificially prolong the remission period (24). While this method may be scientifically sound, it may result in greatly enhanced CR duration figures. We, therefore, believe that there are few studies in which the median duration of remission can be fairly compared to the present study. In ALGB a relapse is determined almost as strictly as in our institution. Remission duration in both arms of the present study compares favorably with that obtained in the most recent studies of that group (26,27).

Patients who were infected on admission and yet achieved a CR had a longer median duration of CR than did patients who were uninfected on admission (8.2 vs 5.2 mos) in the daunorubicin-alone induction arm. It is tempting to speculate that infection, when not lethal, in these patients played a nonspecific immunotherapeutic role such as that ascribed to bacillus Calmette-Guérin (BCG) (28-30).

The wbc count before treatment had an influence on the duration of CR. In both induction-therapy groups, patients whose initial wbc counts before treatment were < 20,000 cells/mm^3 had longer median durations of remission than did those patients whose wbc counts before treatment were > 75,000 cells/mm^3. This effect was most marked in patients

treated with daunorubicin alone. In those patients, the median duration of remission in the low wbc count group was more than twice as long as for patients in the high wbc count group.

The age at diagnosis affected survival in this study. In both induction-therapy arms, patients < 60 years of age had a significantly longer median survival time than did the patients > 60 years. In the combination-therapy group, all the treated patients < 60 years had an 8.2-month median survival whereas older patients had a 2.5-month median survival. In the single-agent group the younger patients had an 11.7-month median survival compared to 1.1 months for the older patients.

Skin-test reactivity of patients prior to therapy in this study was not helpful in predicting response to therapy. No data on serial skin testing in these patients are available and it may be that conversion from skin-test negativity to positivity, as revealed by serial skin testing, may be a more accurate indicator of the patients' ultimate response to antileukemic therapy (31).

Pyrimethamine was used in this study as potential prophylactic therapy for meningeal leukemia. Its lack of effectiveness which is shown in this study argues against continued investigation of this agent for the therapy of meningeal leukemia in patients with ANLL.

Marrow morphology affected the response to therapy in both treatment groups studied. The diagnosis of AMML was associated with a higher CR rate and a longer median duration of survival in both treatment groups. These data are consistent with previous data from this institution (32).

Marrow differentiation prior to therapy did not appear to affect response to therapy in either treatment group. These results differ from those of Brincker (20) and the reasons for these differences are not clear.

Four patients in this study developed daunorubicin cardiac toxicity and all were treated with daunorubicin alone. The patients with daunorubicin cardiotoxicity received significantly more daunorubicin as a group than those patients who showed no cardiac toxicity. Although 27% of the patients who received > 540 mg/m^2 of daunorubicin initially as a single agent developed cardiac toxicity, none of the eight patients who received that total cumulative dose of daunorubicin in combination with other antileukemic agents had cardiac toxicity.

Although cardiac toxicity from daunorubicin is a late drug effect, it is of increasing importance to ANLL patients because of the relatively long survival that results from present-day therapy. If methods could be found to reduce this complication without reducing antileukemic activity, significant improvement in the management of acute leukemia patients would probably result. Early data on complexing the drug with DNA suggest that further exploration of this approach may be warranted. Cornu et al (33) tested daunorubicin-DNA in a group of 17 patients with ANLL. While the CR rate was 53% in that study, only one of the four patients > 60 years of age achieved CR. The CRs were short (median, 4 mos), and the therapeutic results are difficult to compare to the present study since vincristine and cytosine arabinoside were given along with lower doses of daunorubicin in the study by Cornu et al. However, it is of interest that no cardiac toxicity has been observed in four patients who received doses of > 600 mg/m^2 of daunorubicin in that study. Longer followup and greater patient accrual to that study will be necessary to fully evaluate the efficacy of daunorubicin-DNA.

This study served as a pilot study for a much larger one performed by the ALGB (27) to be reported separately in detail. In the ALGB study, a 42% CR rate (49% CR + PR) was obtained with daunorubicin in the same dose and schedule used in the present trial and a 50% CR rate (57% CR + PR) was obtained with a combination of daunorubicin, cytosine arabinoside, and 6-thioguanine given in the same schedule, but at doses augmented by one third over those used in the present study. Since the CR rate for the combination in the ALGB study was greater than in the present investigation (50% vs 46%) and since PRs were less frequent in the ALGB study than in the present one (7% vs 12%), it is possible that the doses of effective drugs in the current study were less than optimal, or that pyrimethamine was deleterious.

There are seven reports (6, 22, 25, 27, 34-36) in which daunorubicin alone was used for previously untreated ANLL in a dose and schedule identical or similar to the plan in the present study. In six of the seven reports, a CR rate of > 40% was obtained and the overall CR rate for the 307 patients in those studies was 47%. When less intensive daunorubicin therapy is used the results are poorer and CR rates of 20% or less are obtained (25, 37, 38). When prednisone is given in addition to daunorubicin in an appropriate dose and schedule, the results are inferior to those obtained with daunorubicin alone. In the six daunorubicin plus prednisone studies (34, 39-43) the CR rate varies from 0 to 26% and the overall CR rate for the 93 previously untreated patients is 22%. Of particular interest is the report by Toghill et al (34) in which five patients treated with daunorubicin alone had a CR rate of 80% whereas none of seven patients treated with the same dose and schedule

of daunorubicin plus prednisone had a CR. Evidence suggests, however, that when cytosine arabinoside is added to a daunorubicin plus prednisone regimen, the possible deleterious effects of prednisone are nullified and CRs of > 40% are possible (44-46). There is no evidence, however, that the addition of prednisone to a drug combination containing daunorubicin is helpful (40).

There are only three published reports on the efficacy of daunorubicin in combination with cytosine arabinoside and 6-thioguanine for the treatment of ANLL (27, 28, 44) and CR rates of 38%-49% are reported. In two of those studies (27, 28) in which patients were randomized to the three-drug combination or a simpler one, the results were slightly better with the three-drug combination. In the study by Vogler et al (28) very small doses of daunorubicin were added to cytosine arabinoside and 6-thioguanine in comparison to the ALGB study (27) and the CR rates were superior in the latter study (38% vs 49%).

In a large number of studies (29,43,47-58), daunorubicin has been combined with cytosine arabinoside, usually in a 5-day course, with daunorubicin given on the first 1 or 2 days and cytosine arabinoside on each of the 5 days (29,43,51-57). The initially high response rate with this scheme (51) has come down to a 42% CR rate in the hands of the original investigators as more patients were treated (29) and results have been even less impressive in larger cooperative trials (55,56). When vincristine is added (49,53) results are not improved nor are they better than would be expected with daunorubicin alone. When daunorubicin is given after cytosine arabinoside (50,58) or when the two drugs are simultaneously administered intermittently (47), early data suggest improved CR rates > 50%. More recently, Yates et al (48) have reported an exceptionally high CR rate (63%) in eight previously AML patients using a 7-day infusion of cytosine arabinoside with daunorubicin given daily for the first 3 days. We have been able to confirm these findings in a larger group of patients. If the early success with that regimen continues at the same level, it will be the first daunorubicin combination to prove significantly more effective than daunorubicin alone for the therapy of ANLL.

It must be emphasized that daunorubicin alone for the treatment of ANLL is at least as productive of CRs as commonly employed combinations of agents such as prednisone, vincristine, methotrexate, and 6-mercaptopurine (1), cytosine arabinoside and 6-thioguanine (27), and cyclophosphamide, vincristine, cytosine arabinoside, and prednisone (24). It is more active than cytosine arabinoside alone (59). However, newer anthracycline combinations such as that reported by Yates et al (48) and an adriamycin, vincristine, cytosine arabinoside, and prednisone combination reported by McCredie et al (60) hold significant promise for improved induction therapy.

The remission duration results in the present study are compatible with those of other studies in which moderately intensive maintenance chemotherapy has been given and regularly scheduled serial bone marrows have been done to assess remission status. They are inferior to studies in which BCG was used during maintenance therapy (28-30) or late intensification chemotherapy was used (61). The precise value of such programs in prolonging remission duration in ANLL is still under investigation.

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Phase II Study of *Cis*-dichlorodiammineplatinum(II) (NSC-119875) in Advanced Adenocarcinoma of the Ovary[1,2,3]

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SUMMARY

Cis-dichlorodiammineplatinum(II) was used in the treatment of 34 patients with advanced adenocarcinoma of the ovary who were resistant to conventional chemotherapy. Two dose schedules were explored: a high-dose schedule with 30 mg/m² given daily on 3 successive days every 4 weeks and a low-dose schedule with 30 mg/m² given once every 2-3 weeks. Nine patients (26.5%) showed a therapeutic response with a median duration of 6 months (range, 3-15 months). The main toxic effects included myelosuppression, renal function impairment, and nausea and vomiting. The high-dose schedule proved to be more toxic without any therapeutic advantage.

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Cis-dichlorodiammineplatinum(II) (*cis*-DDP) was first introduced as a possible anticancer drug in 1965 by Rosenberg et al (1). It has been shown to have antineoplastic activity in many in vitro and in vivo animal systems. In man, therapeutic usefulness has been claimed in cases of testicular tumors, thymoma, lymphoma, and occasional cases of squamous cell carcinoma (2). In 1972 we began a phase II study into its possible usefulness in patients with ovarian adenocarcinoma where the tumor had failed to respond or had relapsed after conventional chemotherapy. Preliminary results have already been published (3). A comparison of two dose schedules was made.

PATIENTS AND METHODS

Thirty-four patients with histologically proven ovarian adenocarcinoma were entered into the study which ended on December 31, 1974; the minimum followup was 4 months. All of the patients had advanced ovarian adenocarcinoma and all had previously been treated with one or more cytotoxic drugs and were considered refractory to conventional chemotherapy. Fourteen of the 34 patients had also received radiation therapy to the abdomen or pelvis. Half of them had been diagnosed for > 12 months and another 25% had been diagnosed for > 24 months before the platinum compound was given. The median age of the patients was 57 years (range, 22-77 years). At the time *cis*-DDP therapy was started all patients had symptoms and measurable signs of advancing tumor.

Treatment Methodology

Cis-DDP was stored as a powder in 10-mg vials and was dissolved in normal saline at a concentration of 1 mg/ml immediately prior to administration. The drug was given as a rapid iv injection. Two different treatment schedules were used: (*a*) schedule A (high dose)—30 mg/m² daily on 3 successive days (these courses were repeated every 4 weeks); and (*b*) schedule B (low dose)—30 mg/m² once every 2-3 weeks. Thirteen patients were treated with schedule A and 22 patients with schedule B. Two patients started on schedule A for two and three courses respectively and were then changed to schedule B. This regimen will be referred to as schedule C.

Since severe vomiting occurred in almost all the patients immediately after each injection of *cis*-DDP, most patients were hospitalized overnight.

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[2]Sponsored by the Swiss and Zurich Leagues against Cancer (Dr. Kroner).

[3]*Cis*-dichlorodiammineplatinum(II): CAS reg. No. 15663-27-1; *cis*-DDP. Supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute.

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Some degree of control of the vomiting was obtained with sedatives and antiemetics, but to avoid dehydration 1-2 liters of normal saline was given over a 12-20-hour period immediately after each injection.

Hemoglobin, leukocyte and platelet counts, and urea and/or creatinine were estimated weekly and prior to each course and the treatment was delayed if the white blood cell (wbc) count was < 1900 cells/μl, the platelet count was < 90,000 cells/ μl, or the blood urea was > 50 mg/100 ml (equivalent to a blood urea nitrogen of 23 mg/100 ml).

Originally, treatment was continued until progression of the tumor became obvious. However, when it was clear that all responders showed some degree of improvement after the first course of treatment, the policy was changed. The patients who were started on treatment in the second year of the study were re-assessed after three courses and treatment was stopped if no improvement was noted at that time.

Definition of Response

For the purpose of this study a therapeutic response has been defined as: (*a*) a decrease in the size of a palpable tumor by > 50% lasting at least 3 months with subjective improvement of the patient; or (*b*) a reduction of ascites and/or pleural effusions needing no further paracentesis for at least 3 months. The duration of response was calculated from the start of therapy. Patients who had signs of progressing disease or no change in signs and symptoms during the time of treatment were considered therapeutic failures. No attempt was made to separate "progressing disease" and "no change" since it is our experience that in ovarian carcinoma, so-called no change usually represents advancing disease.

RESULTS

The number of courses of treatment, the duration of treatment, and the total drug dose given with the different treatment schedules are shown in table 1.

Therapeutic Effects

Response.—Nine of the 34 patients (26.5%) showed a therapeutic response. Table 2 gives details of these nine patients who responded. The best response was seen in patient No. 6 who had a complete regression of a large pelvic mass which lasted for 15 months. Good partial regressions were obtained in four patients with > 75% reduction of tumors and reduction of effusions without further tapping. In four patients the masses regressed < 75% but > 50%. The median duration of response was 6 months (range, 3-15 months). All responders showed some degree of improvement after the first course of treatment. The effect of the drug on tumors in different sites is given in table 3. Both pleural and peritoneal effusions responded as well as abdominal and extra-abdominal masses. Previous local radiation therapy did not prevent regression of tumors.

Failures.—Twenty-five patients (73.5%) showed no therapeutic response to the *cis*-DDP treatment. Included in this figure are three patients who died within 2 weeks of the first course. All of these three patients had been terminally ill with a rapidly advancing tumor before starting treatment. There was no evidence that drug toxicity shortened their lives. Neither the age of the patients nor the length of the course of their disease before treatment seemed to influence the response to *cis*-DDP.

Survival.—The median survival time (MST) was 12 months (range, 7-14 months) after the start of the *cis*-DDP therapy for the nine patients with a therapeutic response, with two patients still alive at 10 and 24 months, both with recurrent tumor. The MST was 6 months (range, 0-23 months) after the start of treatment for the 28 nonresponding patients, with two patients alive at 6 and 9 months. Table 4 shows the therapeutic results for the different regimens. There is apparently no difference

TABLE 1.—*Cis*-DDP: No. of courses, total drug dose, and duration of treatment with different treatment schedules

Schedule	No. of patients treated	Mean No. of courses per patient (range)	Mean total dose in mg per patient (range)	Mean duration of treatment in days per patient (range)
A	13	3.8 (2-7)	400 (160-610)	77 (29-144)
B	19	6.9 (2-17)	310 (80-765)	99 (15-245)
C	2	10 (6-14)	520 (420-620)	246 (200-293)

TABLE 2.—Characteristics of responding patients

Patient No.	Age (yrs)	Prior therapy	Extent of disease	Response to *cis*-DDP: Type*	Response to *cis*-DDP: Duration of remission (mos)	Schedule	No. of courses	Total dose (mg)	Duration of therapy (days)	Survival after *cis*-DDP started (mos)
1	58	Cyclophosphamide 5-Fluorouracil Chlorambucil	Abdominal mass Recurrent ascites Pleural effusions	↓50% 0 isq	3	A	5	396	125	8
2	54	Radiation therapy Cyclophosphamide	Lung deposits Abdominal mass Supraclavicular nodes	↓>75% 0 0	6½	A	5	610	144	12
3	62	Radiation therapy Chlorambucil	Mass right iliac fossa Edema right leg	↓ 50% 0	10	C	14	620	293	24†
4	58	Chlorambucil	Abdominal mass	↓ 50%	6	B	16	720	232	11
5	35	Actinomycin D 5-Fluorouracil	Mass right breast Axillary nodes Pleural effusions Ascites	0 ↓ 50% left:0 right:↓>50% 0	3	A	4	500	98	7
6	49	Radiation therapy Chlorambucil	Pelvic mass Partial intestinal obstruction	0 0	15	B	11	440	212	18
7	59	Cyclophosphamide	Mass right iliac fossa	↓>75%	3	B	9	510	173	10†
8	57	Chlorambucil	Ascites	0	4	B	13	625	194	8
9	51	Chlorambucil Cyclophosphamide	Epigastric mass Axillary nodes	↓ 50% ↓ 50%	8	B	17	765	245	15

*0 = sign disappeared; isq = sign was unchanged;↓ = sign improved.
†Patient alive as of Dec 31, 1974.

TABLE 3.—Effect of *cis*-DDP on ovarian tumor in various sites

Tumor site	No. of patients	No. of responses
Abdomen, pelvis	30	6
Lungs, breast, skin	3	2
Liver	1	0
Axillary/supraclavicular lymph nodes	6	3
Ascites	16	3
Pleural effusions	6	2

in therapeutic efficacy as far as the response rate is concerned. The median duration of response was 3 months (range, 3-6.5 months) with schedule A and 6 months (range, 3-15 months) with schedule B. The difference between the median duration of response is not significant ($P > 0.1$).

Toxic Effects

The most significant toxic effects observed were nausea and vomiting, myelosuppression, and nephrotoxicity. Of the 34 patients three were excluded from the assessment of hematologic and renal toxicity: these patients died within 2 weeks after the first course of treatment.

Nausea and vomiting.—All patients complained of nausea and almost every patient experienced vomiting starting within 1 hour after the *cis*-DDP injection and lasting up to 1 week (but usually no longer than 24 hours). Antiemetics were of little help and it became routine to give large doses of sedatives and iv fluids in order to avoid dehydration, thus necessitating admission of the patients to the ward for 24 hours after the injection.

Hemoglobin.—The most striking feature of hematologic toxicity was a marked fall in the hemoglobin level during the *cis*-DDP treatment. Eighteen of the 31 patients showed a reduction of the hemoglobin level by 1.5 g/100 ml or more. Thirteen of these 31 patients sustained a fall of > 2 g/100 ml. The high-dose schedule A seemed to be more toxic than the low-dose schedule B. The mean fall in hemoglobin in schedule A was 2.4 g/100 ml compared with a 1.7 g/100 ml fall in schedule B. The difference is not significant, however.

Leukocytes and platelets.—Some degree of leukopenia and thrombocytopenia occurred in almost all patients. Two patients had a wbc count of < 1300 cells/μl (minimum 12 cells/μl) at least once. A thrombocytopenia with a nadir of <100,000 cells/μl developed in 21 patients with a nadir of <50,000 cells/μl (minimum 19,000 cells/μl) occurring in five. The mean fall of leukocytes and platelets during a course of treatment was more marked with the high-dose schedule A (table 5). Leukopenia and especially thrombocytopenia often led to a delay of the next course of treatment. This was the case especially in patients who had been previously treated with many other cytotoxic agents or had undergone extensive radiation therapy. There were no clinical complications due to leukopenia or thrombocytopenia but in one patient *cis*-DDP treatment was abandoned because of recurrent prolonged myelosuppression with each dose. This patient had been treated over the previous 3½ years with continuous chlorambucil.[5]

Nephrotoxicity.—Seven patients showed a rise of blood urea to levels between 50 and 75 mg/100 ml during or after the *cis*-DDP treatment. The part played by *cis*-DDP toxicity in these patients is difficult to assess since other factors (such as a urinary tract obstruction due to a large tumor in the abdomen) may have played a role. However, in two additional patients the blood urea rose to > 100 mg/100 ml and this increase was attributed to *cis*-DDP. Both of these patients had been treated with the high-dose schedule A. The first patient showed a blood urea of 100 mg/100 ml after the third course at a total dose of 400 mg in 87 days. The drug was discontinued and 2 months later the urea had decreased to 70 mg/100 ml. The patient died of progressing tumor 3 months after the *cis*-DDP treatment was discontinued. This patient had been treated as an outpatient and had had severe vomiting after the *cis*-DDP injections. Thus, dehydration may have accentuated the renal toxicity of the drug. The second patient had had five courses of *cis*-DDP at a total dose of 610 mg in 144 days. At the time of the last course her blood urea was 50 mg/100 ml. Two months later her blood urea was 120 mg/100 ml. An urography showed no evidence of urinary tract obstruction and a renal biopsy failed to reveal any tubular or glomerular damage. Subsequently, the renal function became worse and the patient died 6 months after the end of the *cis*-DDP treatment with renal failure and progressing tumor. Unfortunately, an autopsy was not done on either of these patients. In contrast to the apparent nephrotoxicity of the high-dose schedule A, the low-dose schedule B proved to be well tolerated even with prolonged application. Five patients on schedule B were treated for > 150 days (median duration of treatment, 212 days; range, 173-245 days) with *cis*-DDP given at a median total dose of 625 mg (range, 440-765 mg). Only

[5]Chlorambucil: NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-.

TABLE 4.—Therapeutic results with different drug schedules

Schedule	No. of patients	No. of responses/ No. of patients treated	Response rate	Median duration of response in mos (range)
A	13	3/13	23%	3 (3-6.5)
B	22	5/19	26%	6 (3-15)
C	2	1/2		10
Total	37	9/34	26.5%	6 (3-15)

TABLE 5.—Hematologic toxicity related to drug schedules

Schedule	No. of patients	Hemoglobin (g/100 ml): mean fall during treatment (range)	Leukocyte count: mean fall during treatment as % of initial value	Platelet count: mean fall during treatment as % of initial value
A	11	2.4(-1.5-4.2)	47.6	53.5
B	18	1.7(0-5.5)	24.9	39.0
C	2	2.6(2.2-3.0)	46.5	55

one of the patients had a rise of blood urea to > 50 mg/100 ml (69 mg/100 ml). None of them had a serum creatinine level of > 1.3 mg/100 ml during or after the *cis*-DDP treatment.

Audiologic toxicity.—Two patients had transient tinnitus but in both cases the audiograms were normal and treatment was continued. No patient complained of hearing loss. Since routine audiometry was not performed this study does not exclude asymptomatic audiologic abnormalities in other patients.

Other observations.—One patient had an attack of seizures at home after *cis*-DDP treatment; the nature and significance of these seizures are questionable.

DISCUSSION

Single-agent chemotherapy with alkylating agents is still the treatment of choice for advanced ovarian carcinoma. Other classes of chemotherapeutic agents and combination chemotherapy have not been shown to exceed the therapeutic effectiveness of alkylating agents (4). Furthermore, our experience with ovarian carcinomas has shown that once resistance develops to an alkylating agent it is extremely rare to achieve further improvement with other drugs. For this reason, we are impressed that with *cis*-DDP therapy we have had nine objective responses out of 34 patients resistant to alkylating agents. The median response duration was 6 months, the longest response lasting 15 months. Of the two treatment schedules, schedule A showed a greater toxicity on bone marrow and kidney function without any therapeutic advantage. In this small series there seemed to be no therapeutic advantage for the high-dose schedule A.

Since *cis*-DDP is a heavy-metal compound, it was feared that long-term application might be accompanied by cumulative kidney toxicity (5). Nevertheless, our low-dose schedule proved to have negligi-

ble measurable kidney toxicity even with prolonged application. As previously observed by Wallace and Higby, renal toxicity of *cis*-DDP seems to be related to high doses given over short periods of time (6), and our two cases of probable renal damage both occurred in patients receiving the high-dose schedule. Nevertheless, it would appear from our observations that therapeutic effectiveness shows no such relation in ovarian adenocarcinoma.

Cis-DDP has many biologic properties similar to alkylating agents (7) such as its effect on DNA synthesis (8) and its cross-linking of DNA (9). Furthermore, the experimental Walker 256 carcinosarcoma with acquired resistance to melphalan and cross-resistance to other alkylating agents is also cross-resistant to *cis*-DDP (7). In view of these experimental findings, it is perhaps surprising that in human ovarian cancer no such cross-resistance occurs. However, a therapeutically synergistic effect of cyclophosphamide in combination with *cis*-DDP has been observed in L1210 leukemia (10) and in Sarcoma 180 (11) in mice, indicating that despite the rather similar biologic activities of the two compounds their effectiveness may be more than additive when used in combination.

It is our opinion that *cis*-DDP is worth further trial in the treatment of ovarian carcinoma. We have therefore started a prospective randomized study in order to compare the value of chlorambucil and *cis*-DDP as single agents in previously untreated cases of ovarian carcinomas and to assess the value of a combination of these two drugs against their sequential use.

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Bleomycin (NSC-125066) Followed by Cyclophosphamide (NSC-26271), Vincristine (NSC-67574), Methotrexate (NSC-740), and 5-Fluorouracil (NSC-19893) for Non-Oat Cell Bronchogenic Carcinoma[1,2]

Victor J. Lanzotti,* David R. Thomas, Paul Y. Holoye, Liam E. Boyle, Terry L. Smith, and Melvin L. Samuels[3]

SUMMARY

The in vivo observation that bleomycin may be used as a synchronizing agent provides the basis for testing 4 days of continuous bleomycin infusion followed by 5 days of intensive chemotherapy with cyclophosphamide, vincristine, methotrexate, and 5-fluorouracil. Thirty-eight patients with extensive non-oat cell bronchogenic carcinoma (adenocarcinoma [17 patients], squamous cell carcinoma [14 patients], and poorly differentiated carcinoma [seven patients]) were registered for chemotherapy. There were 11 patients with 50% regression of all measurable lesions and four with improved but poorly measurable radiographic lesions, providing a crude response rate of 39% (15 of 38 patients). An overall survival median of 19 weeks compares favorably with Veterans' Administration Lung Cancer Study Group control data, but was not substantially better than our own historical controls ($P = 0.15$). The median survival for responders was 36 weeks compared to 16 weeks for historical controls ($P = 0.001$) and 12 weeks for nonresponders ($P < 0.001$).

[Cancer Treat Rep 60:61-68, 1976]

The in vivo observation has been made that melanoma cells manifest enhanced labeling with ^{3}H-thymidine from 48 to 72 hours after 4 days of continuous bleomycin infusion (1). Augmentation of cells in S phase of the cell cycle was hypothesized to represent synchronization by reversible blockade at the S-G_2 boundary. The preceding observation provides a basis for the hypothesis that 4 days of continuous bleomycin infusion followed by 5 days of intensive chemotherapy with cyclophosphamide, vincristine (Oncovin), methotrexate, and 5-fluorouracil (bleomycin-COMF) would allow enhanced chemotherapeutic effect.

MATERIALS AND METHODS

Thirty-eight patients with non-oat cell bronchogenic carcinoma (adenocarcinoma [17 patients], squamous cell carcinoma [14 patients], and poorly differentiated carcinoma [seven patients]) were consecutively registered for chemotherapy with bleomycin-COMF from March 22 through July 31, 1973. All patients had extensive disease (Veterans' Administration Lung Cancer Study Group criteria) (2) and were accepted irrespective of general condition or nutritional status.

Bleomycin was administered as a constant infusion on Days 1-4. The dosage was 30 units/day except for 17 patients with prior radiotherapy, with severe chronic pulmonary disease, or >60 years of age who received 15 units/day. In all patients bleomycin was reduced to 7.5 units/day beginning with the fifth cycle of chemotherapy. The COMF portion of chemotherapy was given on Days 6-10: cyclo-

[1]Received July 28, 1975; revised Nov 21, 1975; accepted Dec 12, 1975.

[2]Bleomycin: [2,4'-bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate.

Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Vincristine: CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Methotrexate: CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

5-Fluorouracil: CAS reg. No. 51-21-8.

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phosphamide (200 mg/m² iv, Days 6-10); vincristine (2 mg iv, Days 6 and 9); methotrexate (20 mg/m² iv, Days 6 and 9); and 5-fluorouracil (450 mg/m² iv, Days 6-10). Chemotherapy was repeated each 29 days. Ten debilitated patients (6.5%-23.7% weight loss) received iv hyperalimentation (IVH) concomitant with chemotherapy (3).

PR was defined as a 50% reduction in the product of two perpendicular diameters of all measurable lesions apparent at or beyond the 29th day of treatment, or a striking improvement in a poorly measurable lesion. Nonresponse was < 50% reduction in any measurable lesion or appearance of a new lesion before 50% reduction of all previously known lesions was obtained. Patients with poorly measurable lesions not showing some improvement were considered nonresponders. There were four patients with improved but poorly measurable lesions: resolution of atelectasis occurred in two, a large pleural effusion without malignant cells cleared in one, and a large apical lung lesion without diameters shrunk considerably in another.

Historical controls included 116 patients with extensive non-oat cell lung cancer (adenocarcinoma [56 patients], squamous cell carcinoma [35 patients], and poorly differentiated carcinoma [25 patients]) palliated with antineoplastic chemotherapy or radiotherapy at the M.D. Anderson Hospital and Tumor Institute from January 1 through December 31, 1972. Controls included responding and nonresponding patients. Forty patients were treated with investigative combination chemotherapy, 15 with palliative radiotherapy alone, and 94 with miscellaneous agents. All histologic and treatment categories within the control population yielded no significant survival differences whether considered individually or after multivariate analysis with other variables (unpublished data).

RESULTS

There were 15 responders to chemotherapy (adenocarcinoma [six of 17 patients], squamous cell carcinoma [five of 14 patients], and poorly differentiated carcinoma [four of seven patients]) among the 38 registered patients (39%). There were eight responders among the 17 patients receiving 15 units/day of bleomycin. Radiographic evidence of response in four patients is shown in figures 1-4. There were no CRs although two near CRs were observed. An overall median survival of 19 weeks compared favorably with the Veterans' Administration Group natural history data but was not substantially better than our own controls (P = 0.15) (fig 5). The median survival of responders (36 wks) was significantly superior to nonresponders (12 wks) and our own controls (16 wks) (P = 0.001, both comparisons) (fig 6).

Several prognosticators were compared for the responders, nonresponders, and 116 historical controls (table 1). There were no obvious differences favoring the responders compared to the nonresponders, except in the category of weight loss prior to therapy. Eight of 14 responding patients compared to only three of 23 nonresponding patients (P = 0.01) had lost <6% of their usual body weight prior to starting chemotherapy. Weight loss was unknown in one patient. Although IVH could not be correlated directly with survival, five of the ten hyperalimented patients losing > 6% of their usual weight responded, as opposed to only two of 17 patients (P = 0.08) with similar weight loss but no IVH. The association of response to chemotherapy with stable body weight and IVH has been previously described (4). All registered women (adenocarcinoma [three patients], squamous cell carcinoma [one patient], and poorly differentiated carcinoma [two patients]) responded to chemotherapy compared to nine of 32 men (P = 0.004).

After an analysis of the first 92 courses of chemotherapy, the following significant toxic effects were observed (table 2). Myelosuppression was associated with 17 episodes of fever requiring antibiotics including four episodes with a positive blood culture. Ten of 92 cycles of chemotherapy were associated with an observed platelet count nadir < 100,000 cells/mm³. No patient required a platelet transfusion, although one developed thrombocytopenia responsive to steroids. Forty-six of 92 cycles of chemotherapy were associated with uncomplicated low-grade fever (100°F-102.8°F) during bleomycin infusion. One episode of fatal pulmonary bleomycin toxicity occurred after a total dose of 120 units of bleomycin. This 55-year-old man had received prior mediastinal irradiation as well as tangential irradiation to multiple rib metastases.

DISCUSSION

The data show that a modest number of patients are responsive to bleomycin-COMF and that these patients realize survival advantage compared to nonresponders and historical controls. Justification for lumping non-oat cell bronchogenic carcinoma patients is due to identical prognoses among histologies, both in our own historical controls and in the treated patients in this report. The response rates were also similar for each of the histologic categories in this study.

Published data on COMF alone (5-7) are not exactly comparable to the present study with respect

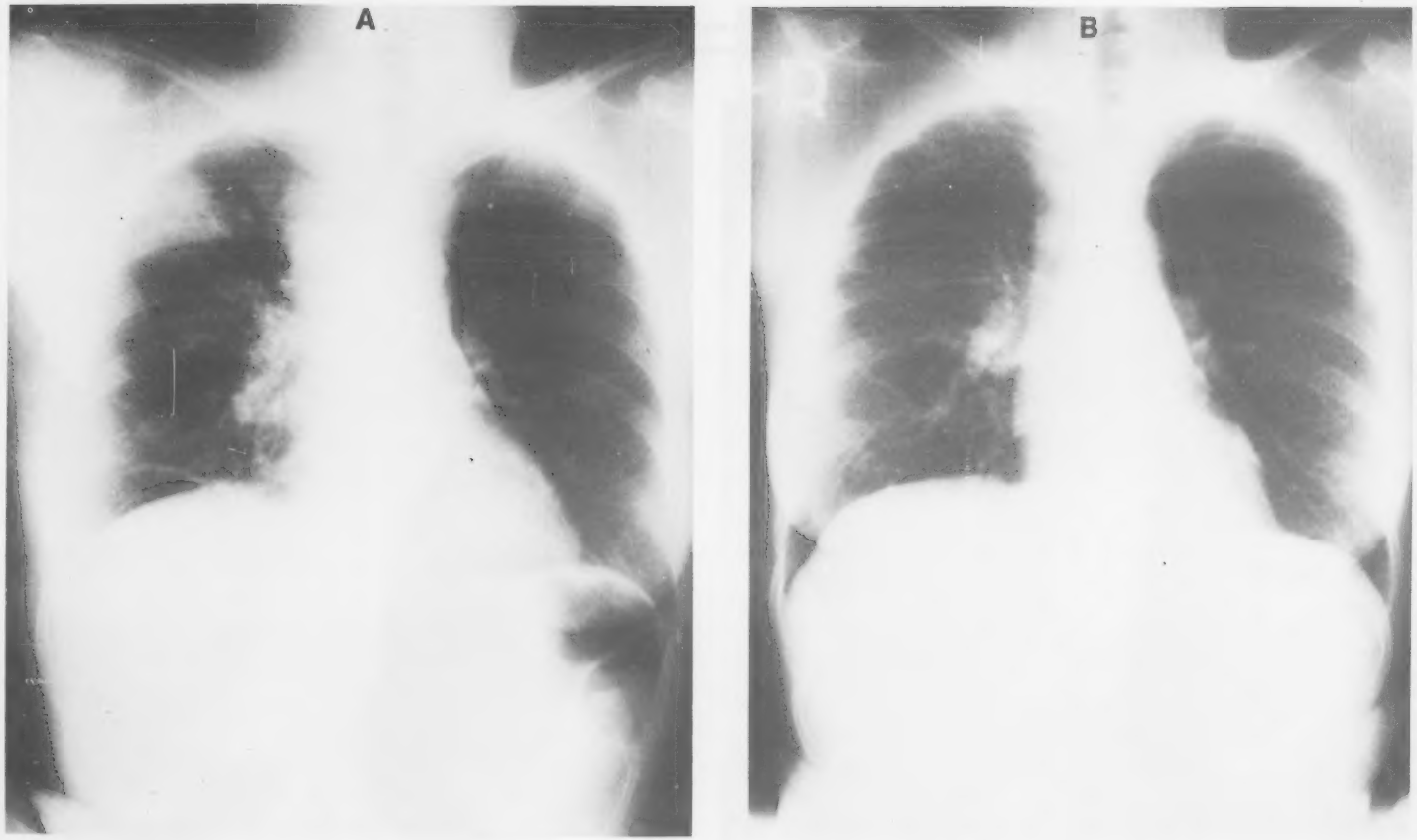

FIGURE 1.—Before therapy (A): A 52-year-old woman with squamous cell carcinoma. Note parenchymal lesions, mediastinal widening, and small right pleural effusion. After therapy (B): Regression in all lesions after 2 courses of chemotherapy. Bilateral supraclavicular lymphadenopathy resolved completely. Survival = 37 wks.

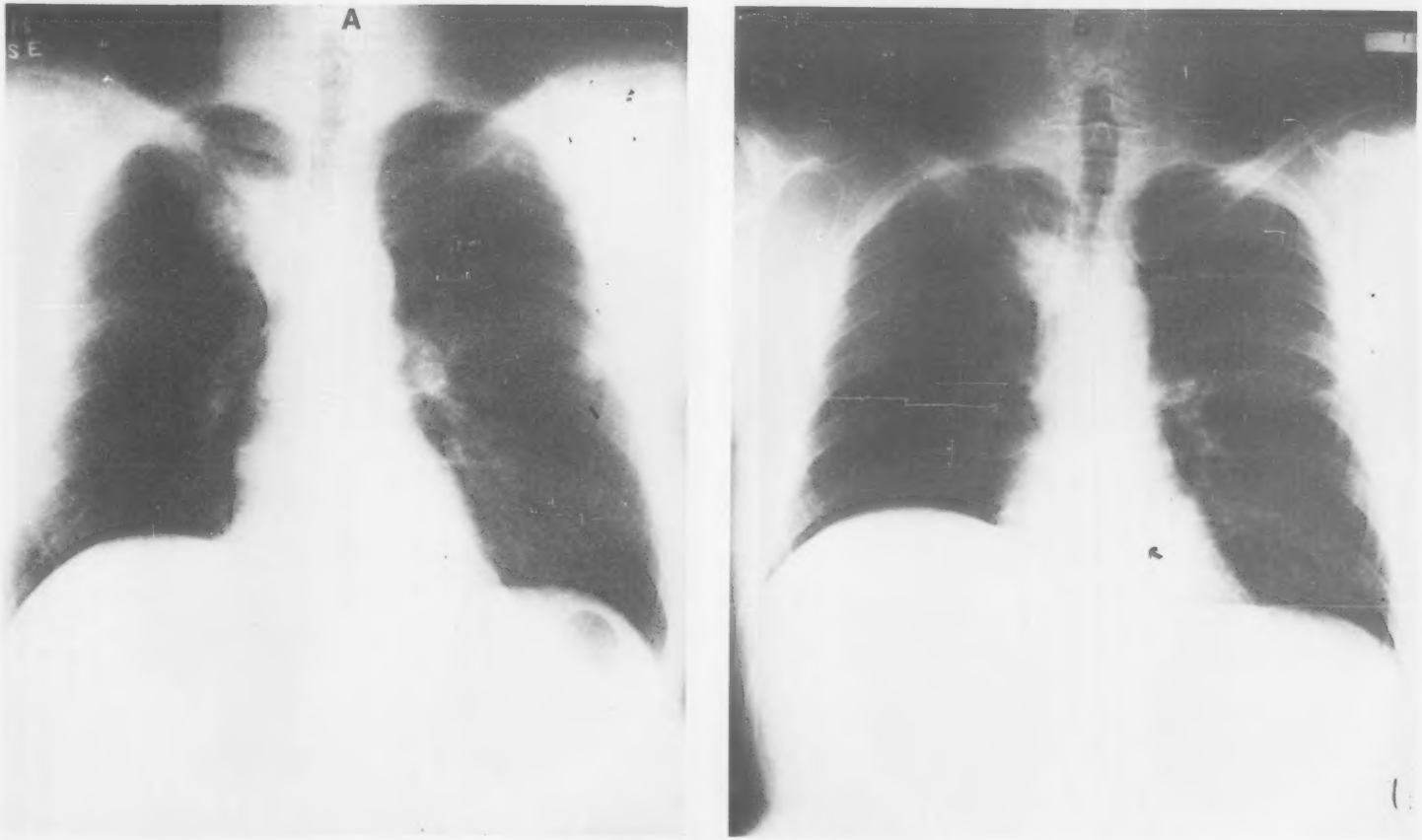

FIGURE 2.—Before therapy (A): A 52-year-old man with bilateral lung lesions of squamous cell carcinoma. After therapy (B): Net regression in all lesions after 3 courses of chemotherapy. Survival = 44 wks.

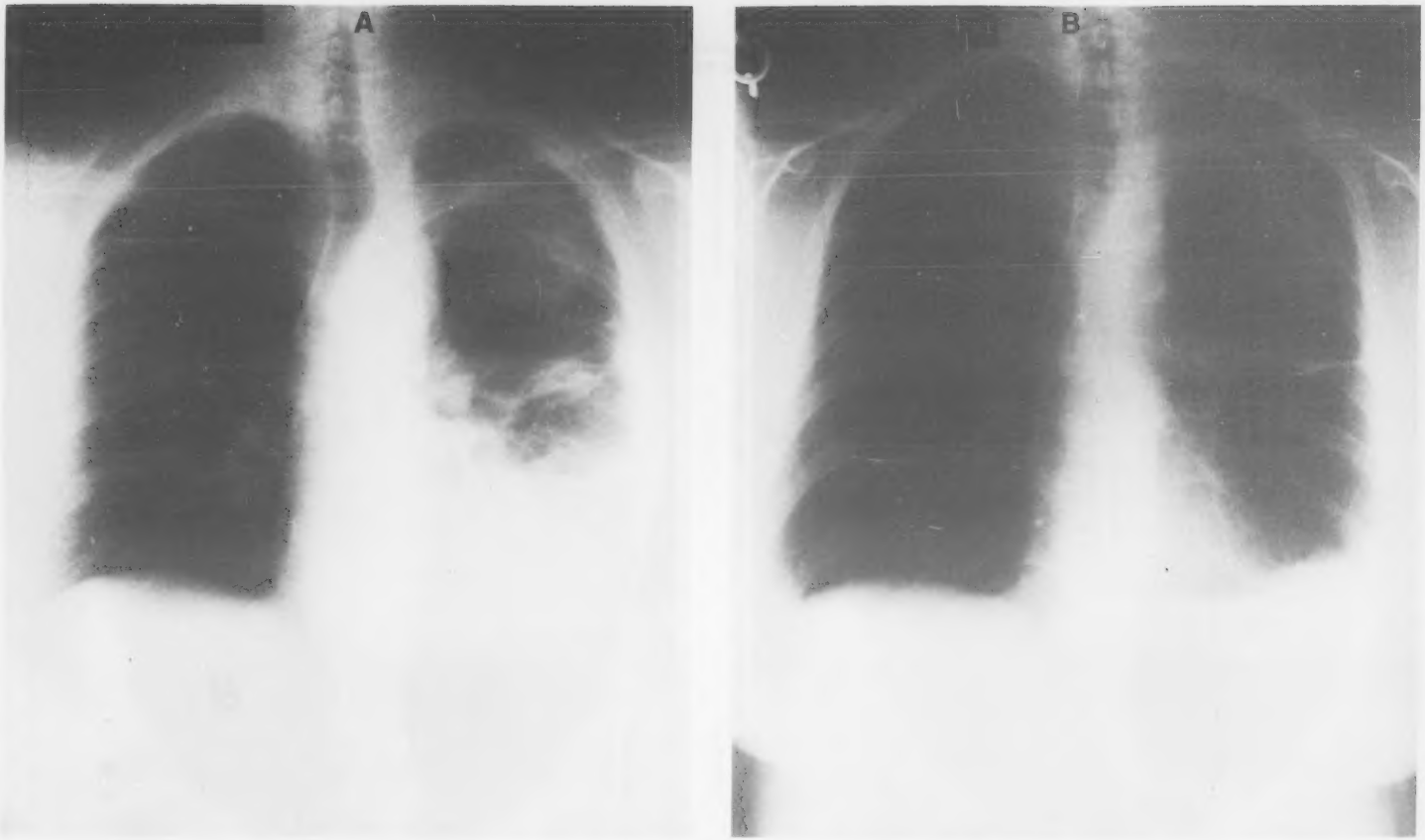

FIGURE 3.—Before therapy (A): A 46-year-old woman with adenocarcinoma. Note parenchymal lesions at left hilum and large pleural effusion. Apparent apical mass is probably due to pleural fluid. After therapy (B): Marked resolution after 3 courses of chemotherapy. Survival = 104+ wks.

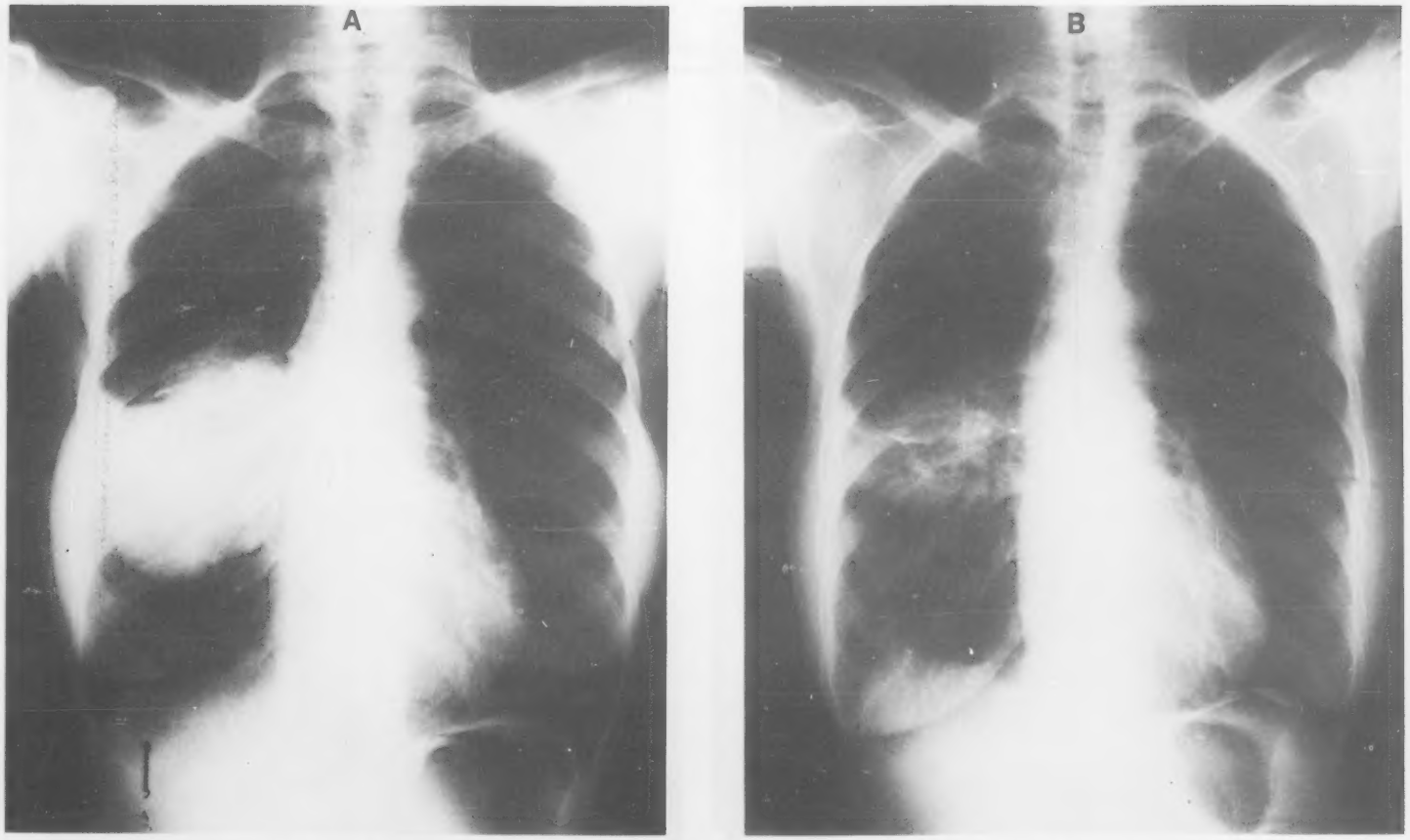

FIGURE 4.—Before therapy (A): A 51-year-old woman with adenocarcinoma weighing 90 pounds. Supported with IVH for 32 days during chemotherapy. After therapy (B): Striking regression apparent after 3 courses of chemotherapy. Nonspecific liver scan abnormalities resolved. Weight increased to 104 pounds. Survival = 72 wks.

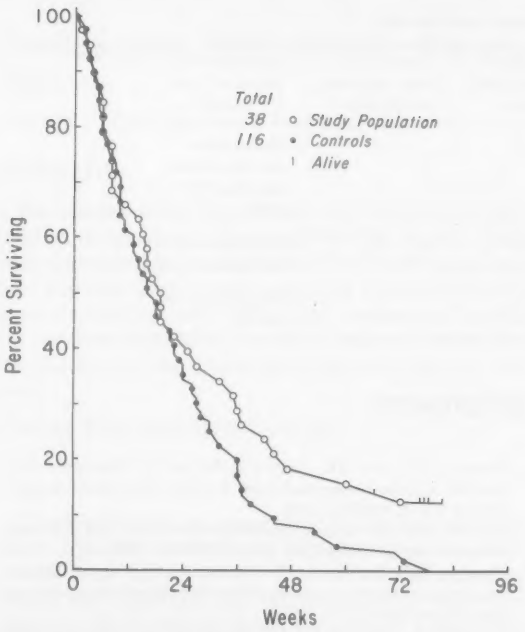

FIGURE 5.—Comparison of survival of all patients treated and historical controls.

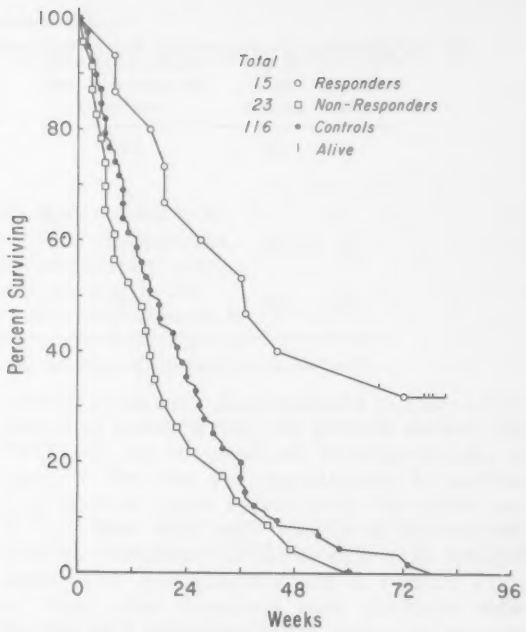

FIGURE 6.—Comparison of survival of responders and nonresponders.

TABLE 1.—Comparison of prognostic variables of responding vs nonresponding patients

	Responders	Nonresponders	Historical controls
Total	15	23	116
Histology			
Squamous cell carcinoma	5	9	35
Adenocarcinoma	6	11	56
Poorly differentiated carcinoma	4	3	25
Performance status*†			
0	0	0	1
1	9	13	80
2	4	6	22
3	0	2	8
4	2	2	5
Weight loss†			
Unknown	1	0	16
<6%	8	3	53
≥6%	6	20	47
Age (≥70)†	1	2	9
Liver involvement†	1	1	15
Brain involvement†	0	0	15
Prior radiotherapy or chemotherapy	5	7	26

*Performance status: 0 = no symptoms, fully active; 1 = has symptoms, fully ambulatory; 2 = bedridden <50% of the time, requires nursing assistance or equivalent; 3 = bedridden > 50% of the time; 4 = bedfast.

†Statistically significant prognosticators within control population after multivariate analysis (unpublished data).

TABLE 2.—Myelosuppression and infection

Course No.	Total No. of patients	Median white blood cell (wbc) count nadir (cells/mm³)	Median wbc count nadir (day)	Episodes of fever requiring antibiotics*	Episodes with positive blood culture
1	38	2050	18	8	2 *(Escherichia coli, Staphylococcus epidermidis)*
2	30	2100	18	5	1 *(Pseudomonas aeruginosa)*
3	24	1800	19	4	1 *(Staphylococcus aureus)*

*Persistent fever >101°F and wbc count <1500.

to the mode of administration, dosage, or scheduling. Studies altering the time sequence and route of administration of the bleomycin and the COMF portions of chemotherapy are currently in progress which will allow further analysis of the synchronization hypothesis. The 1-day rest interval followed by 5 days of COMF represents an arbitrary attempt to time chemotherapy with COMF when relatively more malignant cells might be engaged in active DNA synthesis. The lack of knowledge of the in vivo kinetics of lung cancer cells makes more than arbitrary scheduling impossible. Nevertheless, the modest gains described for responding patients provide the basis for further exploration of bleomycin as a synchronizing agent. The impracticalities of the bleomycin infusion and lack of substantial prolongation of survival for the entire study population preclude routine use of the cited bleomycin-COMF chemotherapy for patients with extensive lung cancer.

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Thalicarpine (NSC-68075): Plasma Decay and Urinary Excretion in Man[1,2]

Patrick J. Creaven[*] and Larry M. Allen[3,4,5]

SUMMARY

The plasma decay and urinary excretion of ^{3}H-thalicarpine were studied in a total of 19 patients at doses of 300-1900 mg/m^2. Plasma decay was triexponential with a terminal phase half-life of 198-1386 hours in five patients. Urinary excretion was slow and erratic, a total of 19.84% of the dose being excreted in 285 hours in nine patients. Only small amounts of unchanged thalicarpine appeared in the cerebrospinal fluid. The data indicate extensive localization of thalicarpine in the tissues and the danger of drug accumulation if it is given at short dose intervals.

[Cancer Treat Rep 60:69-75, 1976]

Thalicarpine is an aporphine benzylisoquinoline plant alkaloid isolated by Kupchan and his associates from the roots of *Thalictrum dasycarpum*, Fisch, and Amé-Lall (1). Its structure is shown in figure 1. Thalicarpine is active against a monolayer culture of KB cells (2) and produces a maximum increase in lifespan of 285% against the Walker 256 carcinosarcoma.[6] It inhibits the incorporation of thymidine into DNA and uridine into RNA and the early steps of nucleotide triphosphate biosynthesis (3). It has recently undergone phase I clinical trial (4) in which it demonstrated major dose-limiting toxic effects on the cardiovascular and central nervous systems. Preliminary reports of its pharmacology in dogs and rats (5) indicated a rapid disappearance from the plasma, but a detailed pharmacokinetic study has not been carried out. We report the results of clinical pharmacology studies carried out in 19 patients receiving the drug as part of the phase I evaluation at doses ranging from 300 to 1900 mg/m^2 of body surface area.

MATERIALS AND METHODS

Thalicarpine was supplied in 100-mg vials which contained thalicarpine and enough solid phosphoric acid and sodium hydroxide to make a solution with a pH of approximately 6 when reconstituted. Thalicarpine specifically labeled with tritium in the 6α and 7 positions (*see* fig 1) was supplied in vials containing 250 microcuries. The material was chromatographically pure when chromatographed in the systems described below and was used as supplied. It was added to the infusion bottle immediately before the beginning of the drug infusion.

The patients treated, their diagnoses, their prior therapy, and the method of drug infusion have been reported previously (4).

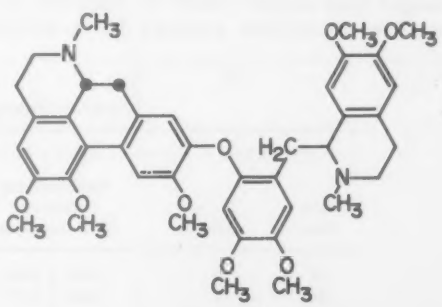

FIGURE 1.—Structure of thalicarpine.● indicates the positions of the tritium label.

[1]Received Aug 18, 1975; accepted Oct 23, 1975.

[2]Thalicarpine: CAS reg. No. 5373-42-2. Both the labeled and unlabeled forms were supplied by Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute (NCI).

[3]NCI-VA Medical Oncology Branch, Veterans' Administration Hospital, Washington, DC.

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[5]The authors wish to thank Ms. C. Williams for expert technical assistance and Ms. B. Nespoli for help in preparation of the manuscript.

[*]*Reprint requests to:* Patrick J. Creaven, MD, Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

[6]Broder LE, and Carter SK. Clinical brochure: thalicarpine (NSC 68075). Prepared by the NCI, Bethesda, Md, 1971.

Measurement of Drug in Plasma and Urine

Total radioactivity in plasma and urine was measured in a Beckman LS-250 liquid scintillation counter by counting 0.1 ml of plasma and urine in 10 ml of toluene containing 100 g of BBS-3 solubilizer (Beckman), 8 g of 2-(4′-*t*-butylphenyl)-5-(4′′-biphenyl)-1,2,4-oxadiazole, and 0.5 g of 2-(4′-biphenyl)-6-phenylbenzoxazole/liter. Unchanged thalicarpine was measured by chloroform extraction of plasma and urine which quantitatively removed thalicarpine without removing any other radioactive material as determined by thin-layer chromatography on silica gel-G in chloroform:methanol (4:1) and in acetone. When it was determined that part of the radioactivity in the plasma and urine was present as a volatile metabolite, presumably water, the following procedure was used for separation of the three fractions of plasma and urine. Total radioactivity was counted as described above. The plasma was then lyophilized, reconstituted to its original volume, and the radioactivity was counted again. The difference is reported as tritiated water. The reconstituted plasma or urine was then extracted with chloroform and the radioactivity removed is reported as unchanged drug. The remaining radioactivity in the aqueous phase is reported as metabolite.

The postinfusion plasma decay of unchanged thalicarpine for five patients was analyzed using the program NONLIN supplied by Dr. C. Metzler of The Upjohn Company, Kalamazoo, Michigan (6), which was run on an IBM 370/165 computer.

RESULTS

The urinary recovery of total radioactivity is low and erratic. The recovery of radioactivity is shown in table 1 and the kinetics of excretion of radioactivity in four patients in whom this was measured for > 20 days is shown in figure 2. It can be seen that radioactivity, the bulk of which is unchanged thalicarpine (table 1), continues to be excreted in measurable amounts for a period in excess of 1 month after the administration of a single dose. Only negligible amounts of thalicarpine radioactivity are found in the feces.

Plasma radioactivity declines slowly. The results from 12 patients at three different dose levels are shown in figure 3. The plasma radioactivity is initially mostly thalicarpine but by 72 hours unchanged thalicarpine represents < 50% of the plasma radioactivity and a substantial percentage of the radioactivity is volatile radioactivity, presumably tritiated water. Metabolite which is not volatile represents 20% of the radioactivity (fig 4). The decay of unchanged thalicarpine in plasma was fitted by nonlinear least squares regression and was found to fit best a triexponential decay equation

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \quad (1)$$

The parameters of the equation for five patients for whom the analysis was carried out are shown in table 2, and the observed and computer-calculated curves for four of the five patients are shown in figure 5. As can be seen from the data in table 2, the terminal half-life is long, with a mean value of 572 hours, and is also extremely variable with a range of 198-1386 hours in these five patients.

In general, the level of radioactivity in the cerebrospinal fluid is about 20%-30% of the plasma radioactivity at 24-48 hours (table 3). Except in one case, however, the bulk of the radioactivity in the cerebrospinal fluid is tritiated water and the levels of unchanged thalicarpine are quite low, ranging between 0 and 10% of the levels of unchanged thalicarpine in plasma at the same time.

Incubation of thalicarpine with plasma at 37°C for 24 hours did not lead to significant release of tritium as tritiated water from thalicarpine. Incubation of tritium-labeled thalicarpine with isolated microsomal rat liver enzyme preparations active in the demethylation of ethylmorphine produced conflicting results, and no clear pattern of metabolism leading to the production of tritiated water emerged.

TABLE 1.—Urinary excretion of ^{3}H-thalicarpine

Time (hrs)	No. of patients	Radioactivity excreted (% of dose ± SD)	% excreted radioactivity (± SD)		
			Unchanged thalicarpine	Metabolite	Water
72	13	13.85 ± 5.83			
265	9	19.84 ± 8.37			
72	4		60.5 ± 3.8	30.6 ± 2.2	8.9 ± 3.9
144	3		56.4 ± 4.4	31.0 ± 12.6	12.6 ± 4.6

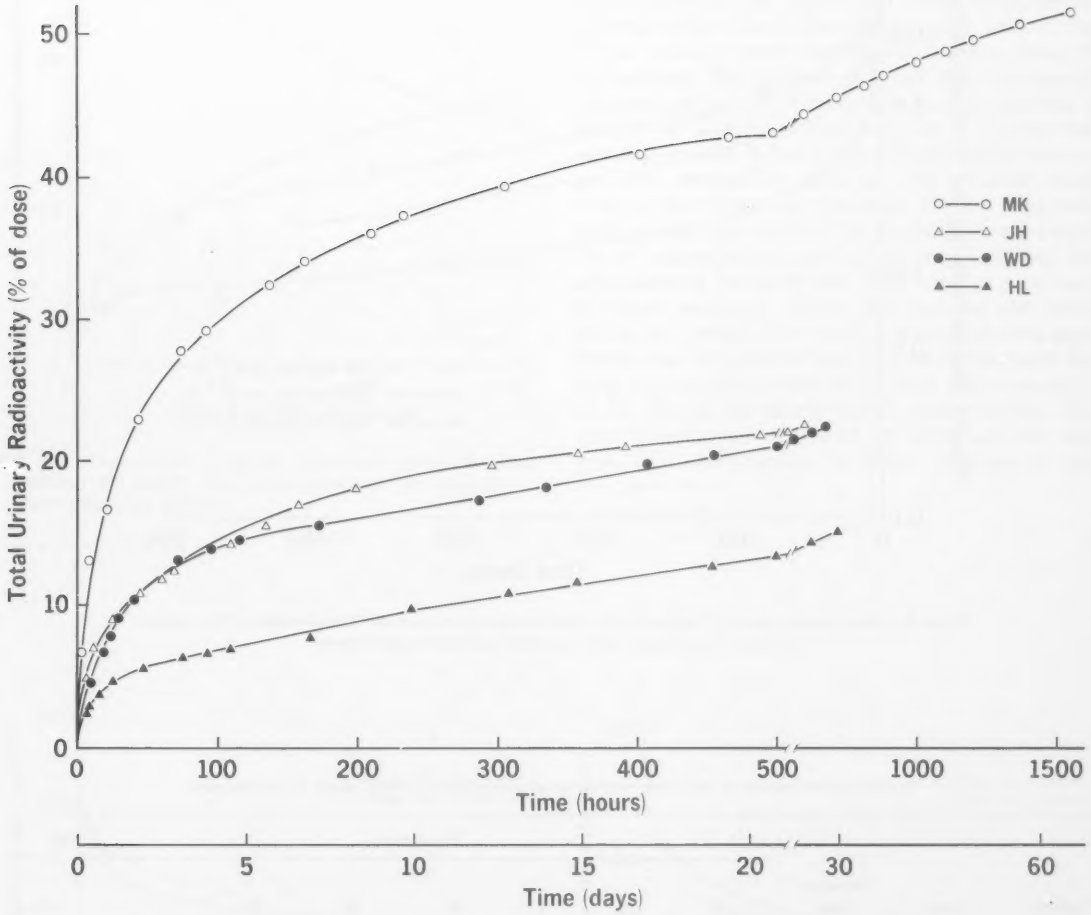

FIGURE 2.—Excretion of total radioactivity by 4 patients after a single iv infusion of ^{3}H-thalicarpine.

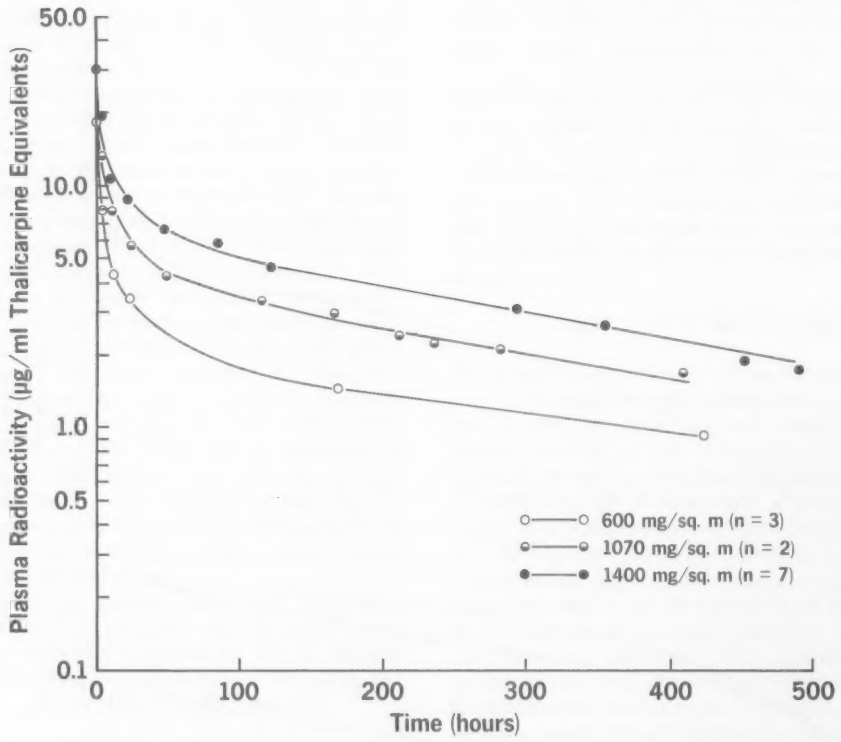

FIGURE 3.—Mean plasma levels of radioactivity after a single iv infusion of ^{3}H-thalicarpine. The number of patients studied at each dose level is given in parentheses.

TABLE 2.—Parameters of nonlinear regression of postinfusion plasma decay of thalicarpine

Patient No.	Dose (mg)	Infusion time (mins)	Parameters							
			A (μg/ml)	B (μg/ml)	C (μg/ml)	α (hr^{-1})	β (hr^{-1})	γ (hr^{-1})	S	Vd_{ss} (liters)
1	2103	120	10.01	3.92	3.46	0.243	0.030	0.0032	1.548 (6 DF*)	447.2
2	2204	120	12.69	5.80	2.32	0.456	0.044	0.0024	1.265 (8 DF)	679.3
3	2355	115	14.99	7.41	2.85	0.516	0.063	0.0009	0.145 (4 DF)	743.3
4	2530	140	35.82	9.69	3.98	1.14	0.073	0.0005	0.036 (4 DF)	599.6
5	2568	120	13.79	12.96	2.66	0.318	0.028	0.0035	0.897 (4 DF)	358.7

*DF = degrees of freedom.

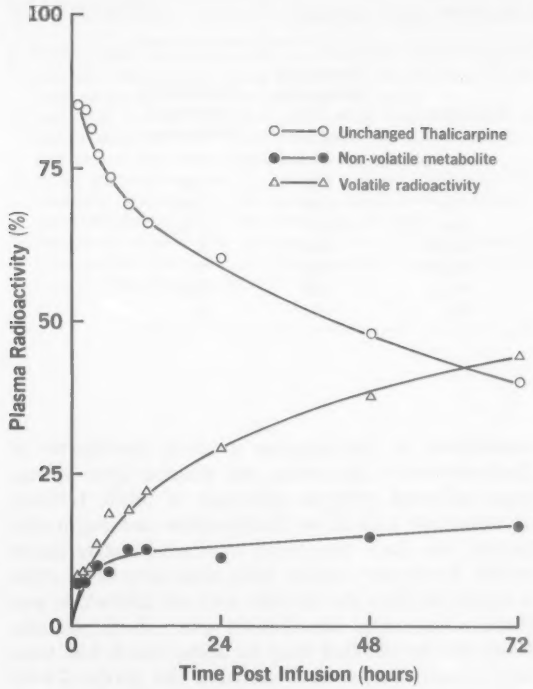

FIGURE 4.—Composition of plasma radioactivity after ^{3}H-thalicarpine (1400 mg/m^2). Each value represents the mean from observations on 4 patients.

DISCUSSION

Thalicarpine is a plant alkaloid of novel structure recently introduced into initial clinical trial (4). Only fragmentary reports of pharmacologic studies in animals are available (5) and these indicate a very rapid plasma disappearance with a half-life of a few minutes. The present study was conducted because of the absence of any information of the clinical pharmacology of thalicarpine and because of the desire to explore schedules of administration other than single dose both in the initial phase I study and in subsequent phase II evaluations. The optimal schedule for therapeutic efficacy against the Walker 256 carcinosarcoma is daily × 5,[7] and we therefore hoped to explore this schedule in man if the data on human pharmacology were compatible with it. The present study shows a very long body retention for the drug with poor overall recoveries. The maximum total recovery of isotope which was achieved was 60% of the administered radioactivity. This was in a patient in whom total collections were carried out for a period of 2 months; the patient was on gastric aspiration and his gastric contents were evaluated for drug over a total period of 22 days (these accounted for 9% of the administered radioactivity). The slow urinary recovery and the long half-life suggest that thalicarpine is either localized in the

[7]See footnote 6.

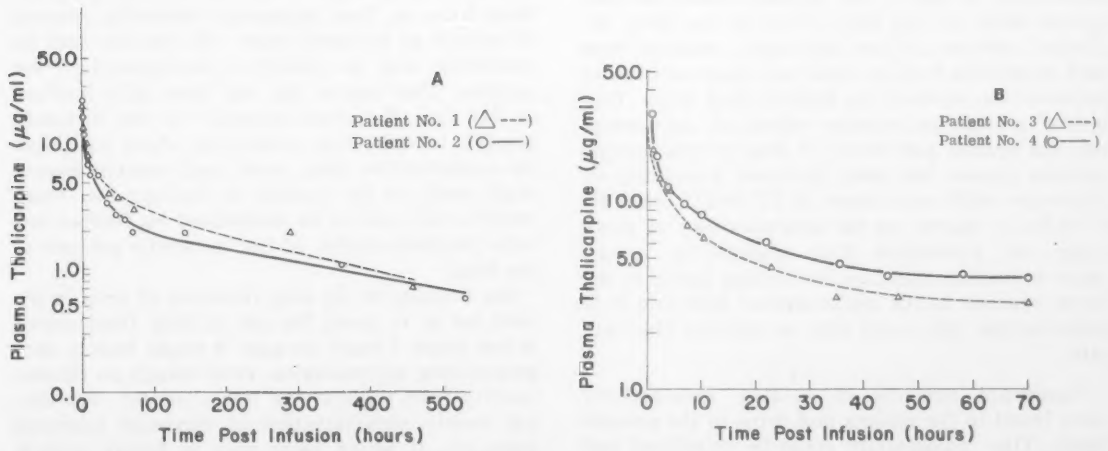

FIGURE 5.—Plasma decay of unchanged thalicarpine for 4 patients. Symbols represent observations. Lines are the best fit of the data to equation 1 by nonlinear least squares regression using the program NONLIN (see Materials and Methods).

TABLE 3.—Thalicarpine in cerebrospinal fluid (CSF) after iv infusion

Patient No.	Time after infusion (hrs)	CSF radioactivity (μg/ml)	% of plasma radioactivity	Unchanged thalicarpine in CSF (μg/ml)	% of plasma unchanged thalicarpine
5	4.65	1.14	4.6	0.32	1.5
5	24.5	2.51	19.62	0.99	9.75
6	6.25	1.61	16.4	0.11	1.48
7	25.0	1.85	20.04	0.00	0
2	25.0	1.8	22.03	0.43	7
8	26.5	3.09	32.39	1.35	9.5
9	46.5	1.63	27.5	0.00	0

body fat or is tightly bound to some macromolecular component of the body tissues. In this connection we found that the plasma binding of the drug is extensive; however, we were unable to demonstrate a significant degree of binding to plasma albumin in vitro and have not so far identified the component of plasma responsible for the plasma binding of the drug. The fact that the plasma decay is triphasic could indicate the presence of a deep compartment to which thalicarpine is very tightly bound and from which it is only very slowly released. This concept is supported by the large steady state volume of distribution (table 2) indicating extensive localization of the drug outside the plasma. In this connection it has been shown that thalicarpine binds to DNA (7).

The presence of only small amounts of unchanged thalicarpine in the cerebrospinal fluid is noteworthy in view of the marked central nervous system effect of the high doses of the drug (4). Central nervous system depression ranging from mild drowsiness to semi-coma was observed in most patients who received the highest drug doses. This lack of correlation between effects on the central nervous system and levels of drug in the central nervous system has been discussed previously in connection with our studies of VP-16-213 and VM-26 (8,9). In reports on the pharmacology of those drugs we postulated that insolubility in an aqueous medium might be the limiting factor in the levels reached in the cerebrospinal fluid and it is possible that this could also be true for thalicarpine.

Significant amounts of volatile radioactivity were found in the plasma and urine in the present study. This radioactivity could be volatilized and recondensed and therefore we assumed it was tritiated water. This could result from spontaneous breakdown of thalicarpine in vivo, breakdown of thalicarpine in the urine and plasma after it had been collected, tritium exchange of labile tritium, or metabolic attack on thalicarpine causing oxidation of the 6α-7 bond with production of tritiated water. From our studies with thalicarpine in vitro it appeared that the tritium was not labile nor was there substantial breakdown over short periods. However, we did find that in urine which had been kept frozen for several months, the levels of tritiated water were much higher than in urine studied immediately after collection. For this reason the results of these retrospective studies have not been included here. Metabolic oxidation at the 6α-7 position would be encouraged by the extra stabilization conferred on the molecule by the formation of a through resonance system (dehydrothalicarpine). However, although our studies of the incubation of thalicarpine with microsomal preparations from rat liver suggested metabolic removal of tritium as tritiated water, the results were inconsistent and no definitive interpretation was possible. This matter has not been fully resolved at this time. For these reasons it is not currently possible to make firm predictions about the possible contributions that renal and hepatic disease might make to the toxicity of thalicarpine. These matters will need to be determined by further and more detailed studies of the metabolic pattern of the drug.

Our findings of the long retention of drug in the body led us to avoid the use of daily thalicarpine in the phase I study because it might lead to dangerous drug accumulation, even though no chronic toxicity from thalicarpine was observed even during weekly administration of maximum tolerated doses (4). It would seem wise in future clinical studies with thalicarpine to use only weekly administration for this reason.

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Combination Chemotherapy With Adriamycin (NSC-123127) and Cyclophosphamide (NSC-26271) for Solid Tumors: A Phase II Trial[1,2,3,4,5]

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SUMMARY

One hundred and nine adult patients with metastatic carcinoma were treated at 3-4-week intervals with a combination of adriamycin (40 mg/m^2 given iv on Day 1) and cyclophosphamide (200 mg/m^2/day given orally in divided doses on Days 3-6). Ninety-two of 96 patients who had an adequate trial (minimum of two courses or progression of disease after one course) had followup observations of tumor sites and were considered evaluable for response. Overall objective response rates by tumor type were as follows: stage III or IV ovarian adenocarcinoma, 61% (14 of 23 patients); endometrial adenocarcinoma, 67% (four of six patients); cervical adenocarcinoma, 33% (one of three patients); prostatic adenocarcinoma, 18% (two of 11 patients); testicular carcinoma, 33% (one of three patients); lung carcinoma, 21% (four of 19 patients); renal adenocarcinoma, 14% (one of seven patients); gastrointestinal adenocarcinoma, 18% (two of 11 patients); melanoma, 25% (one of four patients); and miscellaneous tumors, no responses in five patients. In patients with ovarian adenocarcinoma who had not previously received any cytotoxic chemotherapy the response rate was 80% (12 of 15 patients) with 33% (five of 15 patients) achieving complete clinical remission. CRs in these patients have now been maintained for periods ranging from 7 to 12+ months. The major toxic effects were mild to moderate leukopenia, alopecia, and nausea with vomiting. Hemorrhagic cystitis was observed in three patients. The combination of adriamycin and cyclophosphamide is an effective treatment for carcinoma of the breast (reported elsewhere), ovary, and endometrium and should be considered for initial chemotherapy in patients with these tumors. Further investigations of its use for melanoma and carcinoma of the lung, prostate, kidney, and gastrointestinal tract are also warranted.

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[2]Presented in part at the 11th Annual Meeting of the American Society of Clinical Oncology, San Diego, Calif, May 7, 1975 (Abstr No. 1179).

[3]Supported in part by Public Health Service grants CA-13612 and CA-17094 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[4]After this study was initiated at the University of Arizona, it was expanded as a pilot study for the Southwest Oncology Group (SWOG) with participation by several member institutions. We would like to thank the following SWOG members for their support and case contribution: *James Cecil* and *Harmon Eyre*, University of Utah Medical Center, Salt Lake City; *John Costanzi* and *Frank Panettiere*, University of Texas Medical Branch at Galveston; *Carl Groppe* and *James Hewlett*, Cleveland Clinic, Cleveland, Ohio; *Barth Hoogstraten*, University of Kansas Medical Center, Kansas City; *Joseph Quagliana*, Southern Nevada Memorial Hospital, Las Vegas; *John Saiki*, University of New Mexico School of Medicine, Albuquerque; and *Edward Weber*, Tumor Institute of the Swedish Hospital Medical Center, Seattle, Wash. We would also like to thank the following Arizona physicians who participated in this study: *Paul Duffey, Morris Fine, Thomas Henry, Ralph Jackson, Nick Mansour, Jr,* and *Ethelann Murray*.

[5]Adriamycin: CAS reg. No. 23214-92-8.

Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

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The combination of the DNA-intercalating antibiotic adriamycin and the alkylating agent cyclophosphamide has been shown to be synergistic in several animal tumors in a variety of doses and schedules (1). We first investigated the use of the adriamycin and cyclophosphamide combination in women with advanced breast cancer and found it to be highly effective with an overall response rate of 78% in 51 patients who had not received prior cytotoxic chemotherapy (2). Encouraged by our initial results in breast cancer we extended our study of adriamycin and cyclophosphamide combination chemotherapy to patients with other types of solid tumors. Our initial results are reported in this paper.

PATIENTS AND METHODS

Adult patients with advanced or metastatic carcinoma (other than carcinoma of the breast) and measurable sites of disease were eligible for this study. Adriamycin (40 mg/m^2) was given iv on Day 1 and cyclophosphamide (200 mg/m^2/day) was given orally in divided doses on Days 3-6. The courses were repeated every 3 or 4 weeks as tolerated. The total adriamycin dose was limited to 480 mg/m^2. Responding patients had therapy continued until this dose limit was reached; subsequent maintenance therapy was not standardized but managed on an individual patient basis.

Blood counts were routinely obtained only prior to each course of therapy. If the white blood cell (wbc) count was < 3000 cells/mm^3 or the platelet count was < 100,000 platelets/mm^3 on Day 22, therapy was delayed for 1 week and thereafter given at 4-week rather than 3-week intervals. If the blood cell counts were persistently low at 4-week intervals, the drug doses were decreased by 25%. A 50% reduction in the initial dose of both drugs was customary for patients > 60 years old and for patients with impaired bone marrow reserve on the basis of prior cytotoxic therapy or extensive involvement of bone by carcinoma. The dose of adriamycin was reduced by 50% for patients with extensive liver disease.

Objective manifestations of disease were assessed at 3-week intervals by physical examinations and measurements of specific disease sites. Special studies such as serum alkaline and acid phosphatase determinations, roentgenograms, tumor- or organ-specific isotopic scans, or sonograms were repeated as indicated at 3-9-week intervals.

An adequate trial of chemotherapy was defined as at least two courses of therapy and survival for at least 6 weeks from the start of therapy, or objective progression of disease after a single course of therapy. Patients who had an adequate trial and followup observations of tumor sites were considered evaluable for response.

A CR was defined as complete disappearance of all evidence of disease for at least 1 month, and a PR was defined as a 50% or greater reduction in the product of the diameters of all measurable lesions for at least 1 month. PR of bone lesions was judged by improvement of symptoms plus either return of elevated alkaline or acid phosphatase values to normal, improvement of bone scan, or recalcification of bony lesions. PR of liver disease was defined as a > 30% reduction in the sum of the measurements of the liver distance below the right and left costal margins and below the xiphoid process, or at least a 50% improvement in the appearance of the liver scan. A PR in patients with abnormalities on indium-111-labeled bleomycin scan attributed to tumor was defined as at least a 50% improvement in these abnormal areas (3). Mixed responses (disappearance of disease at some sites with stable or increasing disease at other sites) were considered treatment failures.

RESULTS

The patient characteristics and the results of treatment are summarized in table 1. The details of response are shown in table 2.

One hundred and nine patients entered this study between February 1974 and June 1975. Ninety-six patients had an adequate trial of chemotherapy. Of the 13 patients who did not have an adequate trial, ten had early deaths (nine due to advanced cancer and one due to drug-related infection [contracted during the second course]), one refused further therapy because of nausea and vomiting, and two were lost to followup after a single course of therapy. Of the 96 patients who had an adequate trial of chemotherapy, 92 had followup observations of tumor sites and were considered evaluable for response to therapy. The other four patients were not evaluable for the following reasons: one was lost to followup without observation of tumor response and three had inadequate data provided to allow determination of response. Sixty of the 92 evaluable patients (65%) had not received cytotoxic chemotherapy prior to entering this study.

Toxic Effects

Combination chemotherapy with adriamycin and cyclophosphamide as given in this study was well tolerated and well suited to outpatient care.

TABLE 1.—Results of adriamycin and cyclophosphamide combination chemotherapy in patients with solid tumors*

Tumor, cell type	No. of patients— Entered	Adequate trial	Evaluable	PR	CR	PR + CR/evaluable (%)
Ovary, adenocarcinoma: Total	28	25[a]	23	9	5	61
NPT	15	15	15	7	5	80
Endometrium, adenocarcinoma: NPT	6	6	6	4	0	67
Cervix, adenocarcinoma: NPT	4	3	3	1	0	33
Cervix, squamous cell: NPT	1	0	0	0	0	0
Testicle, embryonal cell	2	2	2	1	0	50
Testicle, teratocarcinoma	1	1	1	0	0	0
Prostate, adenocarcinoma: Total	13	11	11	2	0	18
NPT	11	9	9	2	0	22
Lung, adenocarcinoma: NPT	7	7	6	1	0	16
Lung, oat cell: Total	7	7	7	2	0	28
NPT	5	5	5	2	0	40
Lung, squamous cell: Total	4	2	2	0	0	0
NPT	3	2	2	0	0	0
Lung, undifferentiated: Total	2	2	2	1	0	50
NPT	1	1	1	1	0	100
Lung, alveolar cell	1	1	1	0	0	0
Lung, mucoepidermoid: NPT	1	1	1	0	0	0
Colon, adenocarcinoma: Total	7	7	7	1	0	14
NPT	3	3	3	0	0	0
Pancreas, adenocarcinoma: Total	4	3	3	1	0	33
NPT	2	2	2	1	0	50
Stomach, adenocarcinoma: NPT	1	1	1	0	0	0
Kidney, adenocarcinoma: NPT	7	7	7	1	0	14
Melanoma	5	4	4	1	0	25
Miscellaneous[†]	8	6	5	0	0	0
Total	109	96	92	25	5	32

*NPT = no prior cytotoxic chemotherapy.

†Includes adenocystic carcinoma of the tongue (1 patient), adenocarcinoma of the parotid gland (1 patient), squamous cell carcinoma of the pharynx (1 patient), carcinoma of the urethra (1 patient), transitional cell carcinoma of the bladder (2 patients), and undifferentiated carcinoma with unknown primary site (2 patients).

TABLE 2.—Clinical characteristics of patients responding to adriamycin and cyclophosphamide combination chemotherapy*

Tumor, cell type	Patient No.	Age, sex	Prior therapy (response)	Disease sites	Response		
					Type	Time to (mos)†	Duration (mos)†
Ovary, adenocarcinoma	1	55, F	None	Abdominal mass, ascites	PR	2	3+
	2	58, F	RT (PR); thioTEPA (PR); chlorambucil (PR); actinomycin D + 5-FU + cyclophosphamide (PR)	Abdominal and pelvic masses	PR	2	6+
	3	49, F	None	Liver (hepatomegaly), pelvic mass, abnormal IB scan	PR	2	8+
	4	54, F	None	Supraclavicular nodes, ascites	CR	1	9+
	5	63, F	RT (PR); chlorambucil (NR)	Abdominal mass	PR	1	4
	6	70, F	None	Abnormal IB scan	PR	2	7
	7	47, F	None	Pleura, ascites	CR	3	7+
	8	47, F	None	Abdominal mass, ascites, abnormal IB scan	PR	1	5+
	9	58, F	None	Pelvic mass	PR	3	4+
	10	54, F	None	Vaginal mass	CR	2	12+
	11	72, F	None	Pelvic mass, abdominal mass (sonogram), skin nodule	CR	1	8+
	12	46, F	RT (PR)	Abnormal IB scan	PR	4	7
	13	61, F	None	Ascites, abnormal IB scan	CR	2	12+
	14	46, F	None	Abdominal mass, liver (scan)	PR	2	3+
Endometrium, adenocarcinoma	15	72, F	RT (? response), progesterone (NR)	Lung	PR	1	4+
	16	60, F	Progesterone (NR)	Lung	PR	2	10+
	17	78, F	RT (PR), progesterone (NR), 5-FU (NR)	Lung, abdominal mass	PR	2	2 (lost to followup)
	18	73, F	RT (pelvis) (NR), progesterone (NR)	Pelvic mass, abnormal IB scan	PR	2	7+
Cervix, adenocarcinoma	19	74, F	RT (NR)	Pelvic mass	PR	1	6
Testicle, embryonal cell	20	21, M	Vinblastine + bleomycin (PR)	Supraclavicular nodes, lung, elevated HCG titer	PR	1	2.5
Prostate, adenocarcinoma	21	67, M	Estrogens (PR)	Bone, elevated alkaline and acid phosphatase	PR	1	3
	22	61, M	RT (NR), estrogens (PR)	Bone, elevated alkaline phosphatase	PR	1	6
Lung, oat cell	23	61, M	RT (brain) (CR)	Lymph nodes, liver, brain	PR	2	4
	24	53, M	RT (PR)	Pulmonary mass, supraclavicular and neck nodes, SIADH	PR	1	6+
Lung, undifferentiated	25	70, F	None	Pulmonary mass, hilar mass	PR	1	10+
Lung, adenocarcinoma	26	45, M	None	Pulmonary, hilar, and chest wall masses	PR	1	5
Colon, adenocarcinoma	27	58, F	5-FU (NR)	Abdominal mass	PR	1	4
Pancreas, adenocarcinoma	28	63, M	RT (brain) (CR)	Supraclavicular nodes, liver (hepatomegaly), brain	PR	1	5
Kidney, adenocarcinoma	29	72, M	None	Bone, skin nodule	PR	1	3.5
Melanoma	30	53, M	BCNU+hydroxyurea+DTIC+BCG (NR)	Lung, skin, liver	PR	1	3+

*RT = radiation therapy; IB scan = ^{111}In-labeled bleomycin scan; HCG = human chorionic gonadotropin; SIADH = syndrome of inappropriate antidiuretic hormone.

†Mos after start of chemotherapy.

Alopecia was observed in all patients. Nausea and vomiting occurred frequently but usually responded to antiemetics; only rarely were they severe enough to warrant discontinuation of therapy. Hemorrhagic cystitis attributed to cyclophosphamide occurred on three occasions and on each occasion was spontaneously resolved without significant associated morbidity.

Myelotoxicity was principally expressed as leukopenia. Thirty-seven of the 109 patients entered into the study (34%) had wbc counts < 3000 cells/mm^3 and six (5%) had wbc counts < 1500 cells/mm^3 at some time during therapy. Although serious infection was rare (two patients [2%]), it was fatal to a 76-year-old man with prior radiation therapy and bone marrow involvement by undifferentiated lung carcinoma who was treated with full doses of adriamycin and cyclophosphamide. Severe leukopenia (wbc count, 400 cells/mm^3) occurred after the second course and the patient contracted pneumonia and died.

Serious thrombocytopenia was rarely seen. Seven patients (6%) had platelet counts < 100,000 platelets/mm^3 and two patients (2%) had platelet counts < 50,000 platelets/mm^3 at some time during treatment. Serious bleeding was not observed and platelet transfusions were not required for any patient.

Cardiac toxicity was not observed. However, to date only six patients have received > 400 mg/m^2 of adriamycin. Thus, the number of patients at risk for dose-related adriamycin cardiac toxicity is still small.

Response

Ovarian adenocarcinoma.—Fourteen of 23 evaluable patients (61%) with stage III or IV adenocarcinoma of the ovary had an objective response to therapy. Two of eight patients who had received prior cytotoxic chemotherapy (25%) had PRs. Twelve of 15 patients with no prior cytotoxic chemotherapy (80%) had objective responses, with five (33%) experiencing CR. (The difference in response between patients with prior cytotoxic chemotherapy and patients without prior cytotoxic chemotherapy is significant [$P = 0.02$].) CRs have now been maintained for periods of 7-12+ months, and no patient achieving CR has yet relapsed.

Uterine adenocarcinoma.—Four of six evaluable patients (67%) with endometrial adenocarcinoma unresponsive to progesterone had objective PRs with adriamycin and cyclophosphamide chemotherapy. One of these responding patients has had continued response of lung lesions for > 10 months.

Cervical adenocarcinoma.—One of three evaluable patients (33%) had an objective PR lasting 6 months.

Testicular carcinoma.—One of two patients (50%) with embryonal cell carcinoma had an objective PR. This patient had previously received intensive combination chemotherapy, making this response particularly notable. The single patient with teratocarcinoma entered in this study (disease metastatic to the lungs and retroperitoneal nodes) had subjective improvement and stabilization of lung metastases during 6 months of therapy.

Prostatic carcinoma.—Two of 11 evaluable patients (18%) with metastatic prostatic carcinoma (all with bone involvement and all refractory to estrogen therapy) had objective regression of bone disease. Neither patient had previously received cytotoxic chemotherapy. Responses were maintained for 3 and 6 months.

Lung carcinoma.—Objective PRs were obtained in four of 19 evaluable patients (21%) with carcinoma of the lung. None of the four responding patients had had prior cytotoxic chemotherapy. Response by cell type is shown in table 1. In patients with oat cell carcinoma who had not had prior chemotherapy the response rate was 40% (two of five patients).

Gastrointestinal carcinoma.—One of seven evaluable patients (14%) with colon cancer had an objective response for 4 months. This patient had previously failed to respond to treatment with 5-fluorouracil alone. One of three patients (33%) with pancreatic carcinoma had an objective response for 5 months. The single patient with gastric carcinoma had no response to adriamycin and cyclophosphamide therapy.

Renal adenocarcinoma.—One of seven evaluable patients (14%) with metastatic adenocarcinoma of the kidney had an objective PR with regression of skin nodules which lasted 3.5 months. (This patient also had lytic bone lesions at the start of therapy but roentgenograms were not repeated to assess response at this site prior to relapse.) Another patient has had stable pulmonary nodules and a stable performance status during 12 months of therapy.

Melanoma.—One of four evaluable patients (25%) with metastatic melanoma had an objective PR. Another patient had a brief mixed response. All four patients had previously failed to respond to intensive combination chemoimmunotherapy which included BCNU, DTIC, hydroxyurea, and bacillus Calmette-Guérin (BCG).

Miscellaneous.—Five evaluable patients with other carcinomas (*see* table 1) had no response to adriamycin and cyclophosphamide therapy.

DISCUSSION

Combination chemotherapy with adriamycin and cyclophosphamide given according to the schedule described in this report is safe and effective and well suited to outpatient administration. Myelosuppression, the major toxic effect in the present study, was comparable to that observed in our previous study (2) and was generally mild to moderate. Excessive hematologic toxicity was observed in only one patient in this study and in two patients in our breast cancer study, and it appeared to be related to impaired bone marrow reserve. We now recommend a 50% reduction in initial calculated drug doses for patients > 60 years old, for patients with impaired bone marrow reserve on the basis of prior cytotoxic chemotherapy, radiation therapy, or extensive bone involvement by carcinoma, and for patients with extensive liver disease. A subsequent dose increase should be attempted if hematologic tolerance is found to be good.

Cardiac toxicity has not yet been observed in the present study; however, only six patients have received total adriamycin doses > 400 mg/m^2. In our prior study of 57 patients with breast cancer, cardiomyopathy manifested as cardiac dilatation and congestive heart failure occurred in two of the 18 patients receiving > 400 mg/m^2 of adriamycin combined with cyclophosphamide (2). Both of these patients received doses of 550 mg/m^2 of adriamycin.

Serial evaluation of ventricular function using systolic time intervals (4) or echocardiography (5) may allow the detection of adriamycin-induced cardiomyopathy before it becomes clinically overt. At present, limitation of the total adriamycin dose to 480 mg/m^2 appears to be an adequate precaution for avoiding clinically significant cardiomyopathy.

The combination of adriamycin and cyclophosphamide appears to be synergistic in some animal tumors (1) and perhaps in some human tumors (6,7). Our own clinical studies indicate that it is an especially effective treatment for advanced carcinoma of the breast (2), ovary, and endometrium.

In ovarian adenocarcinomas, response rates ranging from 31% to 64% have been reported for single alkylating agents (including cyclophosphamide) (8). A response rate of 25% has been reported for adriamycin as a single agent (9). The objective response rate of 80% that we observed with adriamycin and cyclophosphamide in the group of 15 patients with no prior cytotoxic chemotherapy is comparable to the best reported response rates for single-agent or combination chemotherapy in ovarian cancer (8). Moreover, one third of our patients achieved CR. Using similar doses of adriamycin and cyclophosphamide, Parker et al (10) have also obtained excellent results in patients without prior radiation therapy or chemotherapy (eight objective responses in ten patients [80%]). Since their initial report, six more responses have been obtained in the next eight previously untreated patients.[8] Therefore, their overall response rate with this combination (78%) is nearly identical to that obtained in our study.

Although the median duration of response cannot yet be determined for our patients with ovarian cancer, the potential for prolonged disease-free survival following induction of CR with chemotherapy has been shown (11). We conclude that for previously untreated patients with ovarian adenocarcinoma the combination of adriamycin and cyclophosphamide is superior to conventional single-agent therapy and probably is the chemotherapy of choice at this time. Moreover, because the response rate with this combination is high in advanced ovarian cancer, it is a logical choice as adjuvant therapy for stages I and II ovarian cancer, where there is a 50% likelihood of recurrence of disease after surgical treatment and radiation therapy (8).

Our preliminary experience, and that of Chia et al (12), suggests that the adriamycin and cyclophosphamide combination has considerable activity against metastatic endometrial cancer. Four of our six patients responded to therapy and Chia et al obtained responses in four of six similarly treated patients. Therefore, eight of 12 patients (66%) with endometrial carcinoma who had not received prior cytotoxic chemotherapy had objective tumor response to this drug combination. Although the number of cases is small, this response rate appears to be substantially better than the response rates achieved with single-agent chemotherapy or with progestational agents alone (13,14). If this preliminary experience is confirmed, adjuvant studies with the combination would be appropriate in patients with an unfavorable prognosis after initial surgical and radiation therapy.

The response of the patient with embryonal cell carcinoma refractory to vinblastine and bleomycin therapy is noteworthy and suggests that the combination of adriamycin and cyclophosphamide with drugs having different toxic effects, such as bleomycin, vincristine, or *cis*-dichlorodiammine-platinum (II), may be worth investigating for testicular carcinoma.

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[8]Tobias J, Griffiths J, Weiner R, et al. Personal communication.

Current chemotherapy for melanoma and carcinomas of the kidney, prostate, gastrointestinal tract, and lung is generally unsatisfactory. Although the rate of response to adriamycin and cyclophosphamide in patients with these tumors was relatively low in this study, we did observe definite activity, especially in patients who had not had prior chemotherapy. We believe that further investigation of this combination as initial chemotherapy should be undertaken in these tumors and in other tumors known to be associated with a low probability of response to other types of chemotherapy. Our evaluation of this combination as initial chemotherapy is continuing.

The high response rates in breast, ovarian, and endometrial carcinoma obtained by us and by others and the considerably higher response rates in patients without prior cytotoxic chemotherapy suggest to us that this combination may now be the treatment of choice for initial chemotherapy for these neoplasms. Moreover, the rationale is clear for extending the use of combinations such as adriamycin and cyclophosphamide that have proved to be highly effective against advanced disease to the adjuvant setting in an attempt to eradicate micrometastases remaining after local initial surgical or radiation therapy.

ADDENDUM

Since submission of this manuscript, another patient with prostatic cancer treated with adriamycin and cyclophosphamide has had a dramatic and nearly complete objective response (documented by bone scan). Therefore, the adjusted response rate in evaluable patients with prostatic cancer is 25% (three of 12 patients).

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Multidisciplinary Curative Treatment for Disseminated Carcinoma of the Breast[1]

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SUMMARY

Eighty-four patients with disseminated breast carcinoma were treated with the combination of hormonal therapy and combination chemotherapy from the time of diagnosis. Hormonal treatment included castration for women still menstruating, estrogens for women menopausal for > 5 years, and androgens or progesterone for women menopausal for < 5 years. Combination chemotherapy consisted of monthly 5-day courses of 5-fluorouracil (500 mg/day, Days 1-5), cyclophosphamide (300 mg/day, Days 1 and 4), vincristine (1 mg/day, Days 2 and 5), and methotrexate (100 mg, Day 1) followed by citrovorum factor (12 mg/day, Days 2 and 3). The overall response rate was 83.3%: 43 CRs (51%) and 27 PRs. The median survival time was 17 months: 6 months for the failures, 12 months for the 27 PRs, and 27 months for the 43 CRs. Fifteen patients with CR are still alive: one after 16 months, one after 17 months, two after 19 months, and one each after 23, 24, 25, 31, 35, 37, 38, 43, 48, 68, and 70 months.

[Cancer Treat Rep 60:85-89, 1976]

Traditionally, disseminated breast cancer has been treated with hormonal therapy, and chemotherapy has been relegated to patients who have failed to respond two or three times to previous treatment. Primary endocrine therapy involves castration in premenopausal women and treatment with estrogens, androgens, or progesterone in postmenopausal women. Secondary endocrine therapy includes adrenalectomy, hypophysectomy, or massive doses of corticosteroids. Chemotherapy is usually started only after one or, more often, two treatment failures.

Since 1968 we have used a multidisciplinary treatment for patients with metastatic breast carcinoma from the time of diagnosis. Previously untreated patients are aggressively treated in a combined modality approach.

MATERIAL AND METHODS

A total of 84 patients with disseminated carcinoma of the breast were studied. All patients were accepted in the study and no patients were rejected, however severe the disease. Forty-four had been treated for the primary cancer by surgery only, and 40 had received surgery and radiotherapy. No information was obtained on the number of invaded lymph nodes.

There were 64 cases of thoracic metastases: lung (16), pleura (14), lung and pleura (11), lung and lymph nodes (five), pleura and lymph nodes (three), lymph nodes (six), lymph nodes and skin (seven), and chest wall and skin (two). There were 13 cases of bone metastases: bones only (eight), bones and skin (two), bones and lung (one), bones and central nervous system (one), and bones and pleura (one). There were four cases of central nervous system metastases and three cases of hepatic metastases with jaundice.

Hormonal Treatment

Hormonal treatment included castration for women still menstruating (19 patients: 15 surgical and four radiotherapeutic); estrogen therapy for women menopausal for > 5 years (diethylstilbestrol, 15 mg/day, or ethinylestradiol, 1.5 mg/day orally); or androgen therapy (calusterone [Methosarb], 200 mg/day) or progestational therapy (medroxyprogesterone acetate, 100-300 mg/day orally) for women menopausal for < 5 years.

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Combination Chemotherapy

We utilized a four-drug combination including 5-fluorouracil (5-FU), cyclophosphamide, methotrexate, and vincristine, given in monthly 5-day courses according to the following schedule:
5-FU: 500 mg/day iv, Days 1-5
Cyclophosphamide: 300 mg/day iv, Days 1 and 4
Vincristine: 1 mg/day iv, Days 2 and 5
Methotrexate: 100 mg iv, Day 1, followed by citrovorum factor, 12 mg/day, Days 2 and 3.

If the patient was in CR after 13 courses, the courses were progressively spaced up to every 6 weeks, every 2 months, and, after 2 years of treatment, every 3 months, which is the most desirable schedule.

TABLE 1.—Summary of results for the 84 patients

No. of patients with—	
Thoracic metastases	64
Bone metastases	13
Central nervous system metastases	4
Liver metastases	3
Overall response rate	83.3%
Median survival	17 mos
No. of CRs	43
Median survival of CRs (15 patients are still living: 1 after 16 mos, 1 after 17, 2 after 19, 1 each after 23,24,25, 31,35,37,38,43,48,68, and 70)	27 mos
Median duration of CR	20 mos
No. of PRs	27
Median survival of PRs (4 patients are still living after 16-31 mos)	12 mos
Median duration of PR	7 mos
No. of failures	14
Median survival of failures	6 mos

RESULTS

For the entire group of patients (table 1) the median survival time was 17 months. The overall response rate was 83.3% (70 of 84 patients). There were 43 CRs[3] with a median survival time of 27 months; 15 patients with CR are still alive after 16-70 months. There were 27 PRs[4] with a median survival time of 12 months. Four patients with PR are still alive after 16-31 months. Fourteen patients did not respond and their median survival time was 6 months. The median duration of response was 20 months for the CRs and 7 months for the PRs. Figure 1 shows the percentage of survivors for the entire group, for the CRs, for the PRs, and for the failures.

TABLE 2.—Summary of results for the 64 patients with thoracic metastases

Overall response rate	84.3%
Median survival	16.5 mos
No. of CRs	34
Median survival of CRs (11 patients are still living: 1 after 16 mos, 1 after 17, 2 after 19, 1 each after 23,31,35,38,43,68, and 70)	27 mos
Median duration of CR	18 mos
No. of PRs	20
Median survival of PRs (1 patient is still living after 16 mos)	10.5 mos
Median duration of PR	6 mos
No. of failures	10
Median survival of failures	6 mos

Thoracic metastases: 64 patients (table 2).—Among the 64 patients, 15 who were premenopausal were submitted to castration (11 by surgical treatment and four by radiotherapeutic treatment). The median survival time was 16 ½ months. There were 34 CRs (53.1%) with a median survival time of 27 months. Eleven patients with CR are still alive, one after 16 months, one after 17 months, two after 19 months, and one each after 23, 31, 35, 38, 43, 68, and 70 months. There were 20 PRs (31.2%) with a median survival time of 10½ months. One patient with PR is still alive after 16 months. Ten patients did not respond and their median survival time was 6 months. The overall response rate was higher for postmenopausal patients (45 of 49, 92%) than for premenopausal patients (nine of 15, 60%). The response according to metastatic site was as follows: lung (14 of 16 patients), pleura (12 of 14), lung and pleura (nine of 11), lung and lymph nodes (five of five), pleura and lymph nodes (two of three), lymph nodes (four of six), lymph nodes and skin (seven of seven), and chest wall and skin (one of two). The median response durations were 18 months for CRs and 6 months for PRs. Figure 2 shows the percentage of

[3]CR = a complete disappearance of the lesions, which is easily observable for the thoracic metastases. For bone metastases CR = disappearance of pain and functional impotence, with a partial ossification.

[4]PR = > 50% response. For bone metastases PR = disappearance of pain and functional impotence but no ossification.

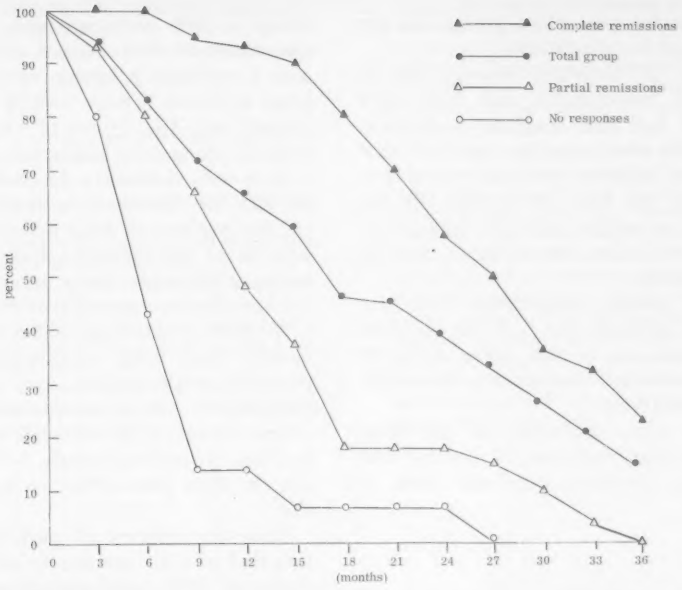

FIGURE 1.—Percent survivors among 84 patients with breast cancer metastases (in mos) for the entire group, for the patients in CR, for the patients in PR, and for the failures.

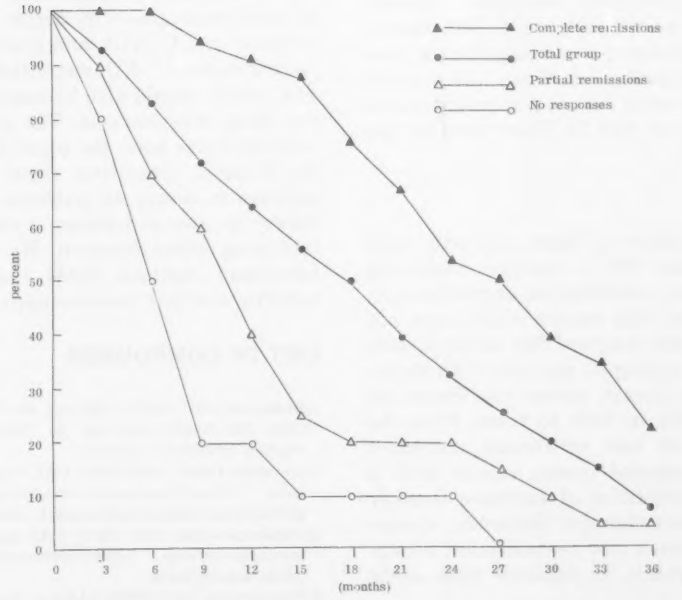

FIGURE 2.—Percent survivors among 64 patients with thoracic metastases (in mos) for the entire group, for the patients in CR, for the patients in PR, and for the failures.

survivors in months for the entire group, for the CRs, for the PRs, and for the failures.

Bone metastases: 13 patients.—Among the 13 patients, nine were menopausal and four were premenopausal and had had surgical castration. There were seven CRs with a median survival time of 22 months. Three patients are still alive, two after 24 months and one after 48 months. Six patients had PR with a median survival time of 15 months: three are still alive, two after 17 months and one after 31 months.

Central nervous system metastases: four patients.—Of the four patients two had CR: one died after 46 months and one is still alive after 37 months. Two patients failed therapy and died after 4 and 7 months respectively.

Liver metastases: three patients.—Of the three patients one had PR (survival time, 18 months) and two failed therapy (median survival time, 3 months).

TOXICITY

Toxicity was quite acceptable and there were no drug-related deaths. Myelosuppression (leukopenia and granulocytopenia) was frequent but of short duration in most patients; anemia and thrombocytopenia were not serious. Alopecia, which occurred in about 50% of the patients, occasionally provoked serious psychological reactions. Nausea, vomiting, and diarrhea were frequent. The vincristine toxicity was significant in seven patients: four had peripheral neuropathy and three had toxicity to the ileus. In these cases vincristine was replaced by vinblastine (5 mg on Day 2). There were no episodes of sepsis.

DISCUSSION

In attempting to achieve a higher response rate, we have studied since 1968 a regimen combining hormone therapy and combination chemotherapy. In previous studies of this nature which were not rigidly outlined we had observed five patients with a > 5-year survival among 31 patients with thoracic metastases from breast cancer (1). Based on this result, we decided in 1968 to treat, from the time of diagnosis, all new previously untreated patients with disseminated breast cancer with a strictly outlined combination of hormone therapy and combination chemotherapy. Recently, Carter (2) and Carbone (3) have also recommended a combined modality approach in patients with early disease.

In the reported literature hormone therapy alone (4-8) has failed to achieve a > 35% response rate. Combined chemotherapy (2-9) alone has achieved about a 55% response rate. By combining these two methods of treatment a higher response rate and a noticeably longer remission duration have been achieved. These results are bringing breast cancer into the group of chemotherapy-sensitive tumors, along with leukemias and lymphomas.

It is still necessary to conduct studies relative to: (*a*) the choice of optimal drug combinations; (*b*) the regimen of drug administration, either continuous or intermittent; and (*c*) the possibility of using a different drug combination after exhausting the effectiveness of the first drug combination.

We have treated our patients with the most frequently used drug combination: 5-FU, cyclophosphamide, methotrexate, and vincristine. Some investigators add systematically prednisone, while others eliminate the vincristine. It appears, however, that cyclophosphamide, 5-FU, and methotrexate are the three most effective drugs in this combination.

Since the efficacy of adriamycin has now been established (10), various trials have combined adriamycin, 5-FU, and cyclophosphamide; adriamycin and vincristine; or adriamycin and CCNU. We feel, as do De Lena et al (11), that it is necessary to study two combinations: (*a*) methotrexate, cyclophosphamide, and 5-FU; and (*b*) adriamycin plus vincristine or adriamycin plus vincristine and CCNU. We plan to administer six to eight courses of adriamycin plus vincristine (or adriamycin, vincristine, and CCNU), and thereafter switch to the combination of 5-FU, methotrexate, and cyclophosphamide. It would also be possible to alternate the two drug combinations. The greatest difficulty encountered has been the psychological effect caused by alopecia (resulting from adriamycin), which appears in nearly all patients. It will also be necessary to choose between continuous or intermittent drug administration. We believe that the intermittent regimen yields superior activity, less toxicity, and less immunosuppression.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Citrovorum factor: NSC-3590; CAS reg. No. 6055-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,-11,20-trione, 17,21-dihydroxy-

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Phase I Clinical Trial of Combined Therapy With Vinblastine (NSC-49842), Bleomycin (NSC-125066), and *Cis*-dichlorodiammineplatinum(II) (NSC-119875)[1,2,3]

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SUMMARY

Seventeen patients with various histologic types of incurable malignant disease were treated with a combination of vinblastine, bleomycin, and *cis*-dichlorodiammineplatinum(II). Creatinine and blood urea nitrogen elevations were noted but were not of a severe degree. White blood cell and platelet count depressions were seen and appeared to be cumulative, though not life-threatening. Tinnitus and high-frequency hearing loss were noted. Tumor regression was seen in one patient with adenocarcinoma of the lung and in one patient with a testicular tumor. This appears to be a manageable drug combination with frequent monitoring of renal, hematopoietic, pulmonary, and auditory function. A phase II study establishing the therapeutic efficacy of this combination in advanced testicular neoplasms now appears to be indicated.

[Cancer Treat Rep 60:91-97, 1976]

In 1965, Rosenberg et al (1) showed that the discharge from an electric current from a platinum electrode through a nutrient broth containing *Escherichia coli* was found to be associated with the inhibition of bacterial replication. Since that time, interest in the platinum coordination compounds as antitumor agents has increased. *Cis*-dichlorodiammineplatinum(II) (*cis*-DDP) has been shown to be one of the most effective agents in this group. In experimental tumors *cis*-DDP has shown activity in the following tumor systems: Sarcoma 180, Dunning ascitic leukemia, Walker 256 carcinosarcoma, mouse reticulum cell sarcoma, 7,12-dimethylbenz(*a*)anthracene-induced rat mammary carcinoma, B16 melanoma, L1210 ascites leukemia, Ehrlich ascites tumor, and Lewis lung tumor (2-9). In man, clinical responses have been noted in the following tumor types: nonHodgkin's lymphoma, sarcoma, Hodgkin's disease, multiple myeloma, and cancer of the breast, testes, ovary, parotid, lung, prostate, thyroid, bladder, and head and neck (6,10-16). In particular, remissions in testicular malignancy have been dramatic, occurring in patients with far-advanced disease who had previously failed multiple therapies.

Dose-limiting toxic effects have been reported to be renal (elevation of blood urea nitrogen [BUN] or creatinine levels, acute tubular swelling) and auditory (tinnitus, high-frequency hearing loss). Myelosuppression has been mild to moderate, therefore *cis*-DDP would be an ideal drug for combination chemotherapy.

Vinblastine (a plant alkaloid) and bleomycin (a soil fungus antitumor antibiotic) have been shown to be individually active in testicular cancer (17, 18).[5] When the two drugs were combined, Spigel and Coltman (19) were able to show superior re-

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[2]Supported by Public Health Service grants CA-07127 and CA-08096 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Vinblastine: CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate.

Bleomycin: [2,4'-bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate.

Cis-dichlorodiammineplatinum(II): CAS reg. No. 15663-27-1; *cis*-DDP. Supplied by Drug Research and Development Program, Division of Cancer Treatment, NCI.

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[5]Agre K. Clinical summary on bleomycin. Bristol Laboratories, Oct 1974.

sults, suggesting therapeutic synergism. Thus, it seemed reasonable to us to combine vinblastine, bleomycin, and *cis*-DDP in a phase I study to determine optimal dose levels and to define the nature and extent of toxicity of this drug combination. This combination might be a highly effective induction-remission scheme for advanced germ-cell malignancy of the testes.

MATERIALS AND METHODS

Patient Selection

All patients who were studied had biopsy-proven noncurable malignancy for which there was no standard therapy available. The patients were informed as to the investigational nature of the study and all gave written consent in accordance with our institutional policy. There was no age restriction. Some additional prerequisites for entrance into this study were as follows: *(a)* the expected survival had to be at least 8 weeks; *(b)* the highest level of previous chemotherapy toxicity must have already been passed; and *(c)* patients had to have an absolute granulocyte count of $\geq$ 2000 cells/mm^3, a platelet count of at least 100,000 cells/mm^3, a BUN level $\leq$ 20 mg/100 ml, a creatinine level $\leq$ 1.7 mg/100 ml, a creatinine clearance $\geq$ 75 ml/minute, and no evidence of obstructive uropathy as determined by iv pyelography.

Clinical characteristics of the 17 patients (12 men and five women) are outlined in table 1. Toxicity data for 4 weeks following one course of therapy were sufficient for evaluability in this study. Sixteen patients were evaluable and one was lost to followup. The patients ranged in age from 20 to 68 years (median, 51 yrs).

Treatment Scheme

The treatment scheme is outlined in table 2. Vinblastine was given on Day 1 and repeated at 14-day intervals. Bleomycin was given twice weekly (15 mg im) until a total dose of 300 mg was reached. *Cis*-DDP was given daily on Days 1-5 and repeated at 28-day intervals. A course of treatment consisted of the administration of all three drugs in sequence. Renal function tests, audiograms, and pulmonary function tests were repeated before each course of *cis*-DDP was given. If the BUN level was $\geq$ 30 mg/100 ml and/or the creatinine level was $\geq$ 2.0 mg/100 ml, or an audiogram showed a deterioration of hearing, *cis*-DDP was to be withheld. The following doses were given (vinblastine and *cis*-DDP = mg/m^2, bleomycin = mg):

two patients—vinblastine (4), bleomycin (15), *cis*-DDP (15);

four patients—vinblastine (6), bleomycin (15), *cis*-DDP (15);

three patients—vinblastine (8), bleomycin (15), and *cis*-DDP (15);

TABLE 1.—Patient characteristics

Tumor category	No. of patients	Previous chemotherapy
Mesothelioma	1	Cyclophosphamide, adriamycin, actinomycin D, vincristine, methyl-CCNU, DTIC
Osteogenic sarcoma	1	Cyclophosphamide, adriamycin, actinomycin D, vincristine, methyl-CCNU, DTIC
Adenocarcinoma, kidney	4	None
Adenocarcinoma, lung	4	1 patient: vincristine, cyclophosphamide, methotrexate
Adenocarcinoma, colon-rectum	3	1 patient: 5-FU, mitomycin C, 5-FUDR
		1 patient: 5-FU, mitomycin C
		1 patient: 5-FU, mitomycin C, 5-FUDR
Squamous cell carcinoma, lung	1	None
Melanoma	1	BCNU, DTIC, hydroxyurea, VP-16-213, cyclocytidine
Carcinoma, testes	1	None
Carcinoma, prostate	1	5-FU, adriamycin

one patient—vinblastine (4), bleomycin (15), and *cis*-DDP (10); and
six patients—vinblastine (6), bleomycin (15), and *cis*-DDP (10).

RESULTS

Toxic Reactions

The toxic reactions that occurred with the combination of vinblastine, bleomycin, and *cis*-DDP are shown in table 3. Nausea and anorexia were found to be the most frequent side effects and at times persisted for up to 10 days following *cis*-DDP. Vomiting appeared to be controlled by antiemetics and did not prohibit adequate oral intake. Serial audiograms did not show any evidence of high-frequency hearing loss. However, this may be because many of our patients failed to receive more than two courses of *cis*-DDP. We did not note any significant rise in serum uric acid necessitating medical therapy.

There was no significant difference in renal impairment among the five different dose ranges (table 4). Earlier reports have noted that both renal and audiologic toxicity appear to be cumulative and may account for the paucity of these findings in our patients.

Because hematologic toxicity was seen we were unable to give vinblastine every 14 days as specified in the original protocol design. However, an every-28-day schedule was manageable. We did not encounter any therapy-related cases of bleeding or sepsis. Tables 5 and 6 summarize the experience of the 16 evaluable patients; there appeared to be some cumulative bone marrow suppression, but adequate recovery occurred allowing further therapy. It might be noted, however, that eight patients had been heavily treated prior to this phase I study and the bone marrow reserve most certainly was compromised and may have accounted for the observed cumulative toxicity.

TABLE 3.—Toxic reactions among 16 evaluable patients

Type	No. of patients
Nausea	10
Anorexia	7
Vomiting	2
Abdominal pain	1
Chest pain	1
Dermatologic	1
Audiologic	0

TABLE 2.—Treatment scheme

Drug	Dose and schedule	Drugs given on day— 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Vinblastine	4, 6, or 8 mg/m² iv, q14d	×														×												×					
Bleomycin	15 mg im biweekly × 10 wks			×		×			×		×							×		×				×		×				×		
Cis-DDP	10 or 15 mg/m² iv daily × 5 days, q28d	×	×	×	×	×																							×	×	×	×	×

TABLE 4.—Renal toxicity

Drug	Dose*	BUN level (mg/100 ml) Mean baseline [No. of determinations] (range)	BUN level (mg/100 ml) Mean peak [No. of determinations] (range)	Creatinine level (mg/100 ml) Mean baseline [No. of determinations] (range)	Creatinine level (mg/100 ml) Mean baseline [No. of determinations] (range)
Vinblastine Bleomycin *Cis*-DDP	4 15 15	15 [2]	18 [2] (7-29)	0.85 [2] (0.7-1.0)	0.85 [2] (0.7-1.0)
Vinblastine Bleomycin *Cis*-DDP	6 15 15	12 [5] (8-16)	15.5 [9] (4-45)	0.83 [5] (0.6-1.4)	1.12 [9] (0.6-2.0)
Vinblastine Bleomycin *Cis*-DDP	8 15 15	11.5 [4] (5-15)	17.7 [8] (13-33.1)	0.90 [4] (0.7-1.3)	1.13 [1] (0.6-1.8)
Vinblastine Bleomycin *Cis*-DDP	4 15 10	7 [1]	7 [1]	1.20 [1]	0.80 [1]
Vinblastine Bleomycin *Cis*-DDP	6 15 10	14 [6] (11-23)	19.5 [9] (8-30.3)	0.88 [6] (0.5-1.3)	1.28 [8] (1.0-1.5)

*Vinblastine and *cis*-DDP = mg/m^2; bleomycin = mg.

Therapeutic Response

Because this was a phase I study we were not particularly looking for antitumor activity. However, in two patients we noted a partial remission (≥ 50% decrease in the products of the greatest diameters of all measurable disease): one patient with adenocarcinoma of the lung (3 mos) and the other with mixed seminoma, chorioteratocarcinoma of the testicle (group 4, according to the classification system of Dixon and Moore). The second patient continues in remission 7 months after therapy was started.

DISCUSSION

Based on the findings of this phase I study, we found the combination of vinblastine, bleomycin, and *cis*-DDP at the dose levels shown to be manageable therapy with no adverse side effects. We feel that vinblastine, however, should be administered at 28-day intervals rather than at 14-day intervals as originally designed. The reason for this can be seen in tables 5 and 6; the nadir of the white blood cell (wbc) and platelet counts frequently occurred so late in the cycle that the additional dose of vinblastine could not be given. Based on our data we recommend the following doses for patients without prior chemotherapy and/or irradiation to major bone marrow producing areas: vinblastine (8 mg/m^2 iv × 1 repeated every 28 days), bleomycin (15 mg im twice weekly [total dose 300 mg]), and *cis*-DDP (15 mg/m^2 iv daily × 5 days and repeated every 28 days). A phase II study is now in order to establish the therapeutic efficacy of this combination in testicular cancer. If activity is confirmed, a phase III study comparing these three drugs to other established treatments, ie, vinblastine and bleomycin, would be indicated. Frequent monitoring of renal, hematopoietic, pulmonary, and auditory function is essential.

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TABLE 5.—Leukopenia

Drug	Dose*		Course 1: No. of evaluable patients	Course 1: Wbc nadir (cells/mm³)	Course 1: Day of nadir	Course 2: No. of evaluable patients	Course 2: Wbc nadir (cells/mm³)	Course 2: Day of nadir	Course 3: No. of evaluable patients	Course 3: Wbc nadir (cells/mm³)	Course 3: Day of nadir	Course 4: No. of evaluable patients	Course 4: Wbc nadir (cells/mm³)	Course 4: Day of nadir
Vinblastine	4	Mean	2	1855	10.5									
Bleomycin	15	Median		1855	10.5									
Cis-DDP	15	Range		1310-2400	2-19									
Vinblastine	6	Mean	4	3450	18	4	2375	12.5	2	9650	16.5			
Bleomycin	15	Median		3550	17.5		1500	10.5		9650	16.5			
Cis-DDP	15	Range		2300-4400	17-21		1200-5300	9-20		2400-15,900	5-27			
Vinblastine	8	Mean	3	4100	19	3	2467	6	2	1400	18.5			
Bleomycin	15	Median		2900	20		2200	5		1400	18.5			
Cis-DDP	15	Range		2800-6600	8-28		2000-3200	3-9		900-1900	13.24			
Vinblastine	4	Mean	1	4400	13									
Bleomycin	15	Median												
Cis-DDP	10	Range												
Vinblastine	6	Mean	6	4667	16	2	2850	15						
Bleomycin	15	Median		4250	16.5		2850	15	1	2800	15	1	2600	17
Cis-DDP	10	Range		2200-7500	10-20		1300-4400	15						

*Vinblastine and *cis*-DDP = mg/m²; bleomycin = mg.

TABLE 6.—Thrombocytopenia

Drug	Dose*		Course 1 No. of evaluable patients	Course 1 Platelet nadir (cells/mm³)	Course 1 Day of nadir	Course 2 No. of evaluable patients	Course 2 Platelet nadir (cells/mm³)	Course 2 Day of nadir	Course 3 No. of evaluable patients	Course 3 Platelet nadir (cells/mm³)	Course 3 Day of nadir	Course 4 No. of evaluable patients	Course 4 Platelet nadir (cells/mm³)	Course 4 Day of nadir
Vinblastine	4	Mean	2	252,500	10									
Bleomycin	15	Median		252,500	10									
Cis-DDP	15	Range		115,000-390,000	1-19									
Vinblastine	6	Mean	4	159,750	21.5	4	252,750	18	2	81,000	14.5			
Bleomycin	15	Median		165,500	19.5		207,500	15		81,000	14.5			
Cis-DDP	15	Range		104,000-540,000	17-30		71,000-525,000	9-34		58,000-104,000	6-23			
Vinblastine	8	Mean	3	288,000	18	3	195,000	19	2	228,500	18			
Bleomycin	15	Median		176,000	20		157,000	24		228,500				
Cis-DDP	15	Range		148,000-540,000	5-28		97,000-330,000	5-28		199,000-258,000	3-33			
Vinblastine	4	Mean	1	130,000	9									
Bleomycin	15	Median												
Cis-DDP	10	Range												
Vinblastine	6	Mean	6	199,000	24	2	190,750	25	1	15,000	29	1	205,000	17
Bleomycin	15	Median		178,500	25.5		190,750	25						
Cis-DDP	10	Range		45,000-377,000	19-28		91,500-290,000	21-29						

*Vinblastine and *cis*-DDP = mg/m²; bleomycin = mg.

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Activity of Anthracyclines Against an Adriamycin (NSC-123127)-Resistant Subline of P388 Leukemia With Special Emphasis on Cinerubin A (NSC-18334)[1]

Brief Reports and Preliminary Communications

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With the demonstration of broad spectrum activity of adriamycin against human tumors and the limitations imposed on its use by cardiotoxicity, there has been intensive investigation of therapeutic and toxicologic properties of anthracycline derivatives. We developed a subline of P388 leukemia resistant to adriamycin both as a tool to study the mechanism of action of adriamycin and to aid in the selection of anthracycline derivatives with unique properties. One of the anthracyclines we evaluated, cinerubin A (1), retained a degree of activity against the adriamycin-resistant subline. Cinerubin A has a number of structural differences in comparison to the structures of adriamycin and daunorubicin (fig 1). Cinerubin A binds to DNA (2) and inhibits DNA-dependent DNA and RNA synthesis (3), This compound also inhibits RNA-dependent DNA polymerase (4) and blocks tumor induction in vivo by an RNA virus (5). Cinerubin A has antitumor activity against a number of transplanted tumor systems (1,6).

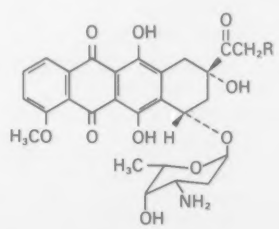
In this report we describe studies of anthracyclines against the adriamycin-resistant subline of P388 leukemia and subsequent studies to determine the activity of cinerubin A relative to adriamycin in a number of tumor systems.

MATERIALS AND METHODS

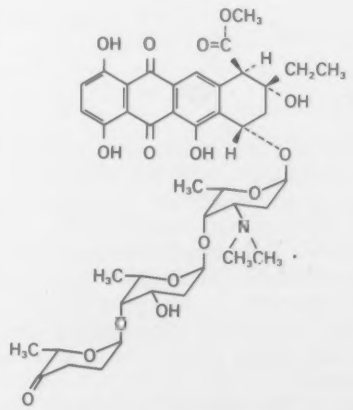
The L1210 and P388 leukemias, B16 melanoma, and Lewis lung carcinoma were maintained in syngeneic mice using the protocols of the DR&DP (NCI) (7). These protocols were also followed for ip implantation of the L1210 and P388 leukemias and B16 melanoma in $B6D2F_1$ male mice for chemotherapy studies. Lewis lung carcinoma was implanted iv as previously described (8).

A subline of P388 leukemia resistant to adriamycin was developed by treating $B6D2F_1$ mice bearing ascitic tumor with adriamycin over a number of transplant generations. A total of 10^6 P388 cells were inoculated ip (Day 0) and mice were treated with 0.65 mg/kg of adriamycin ip on Days 4-12. Tumor cells were collected for subsequent transplant between Days 13 and 18. After eight transplant generations, the treatment schedule was changed to Days 1-9 with transplantation on Day 10. Resistance to adriamycin was complete on the 12th transplant generation.

Drugs were dissolved in sterile water and stored in amber bottles for up to 9 days at 4°C. Drugs were administered in a volume of 0.01 ml/g of body weight by the ip route in all experiments except in



Adriamycin R=OH
Daunorubicin R=H



Cinerubin A

FIGURE 1.—Structural differences of cinerubin A, adriamycin, and daunorubicin.

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TABLE 1.—Effects of anthracycline derivatives on the lifespan of mice bearing P388 leukemia (P388/S) or an adriamycin-resistant subline of P388 leukemia (P388/ADR)

Experiment No.	Drug	Treatment schedule (days)	Dose range (mg/kg/injection)	P388/S		P388/ADR	
				Optimal dose	%ILS*	Optimal dose	%ILS*
1	Adriamycin	1,5,9	2.88-13.33	2.38	173	2.88	18
	Daunorubicin	1,5,9	2.88-13 33	2.88	142	4.8	18
	Cinerubin A	1,5,9	2.88-13.33	4.8	240	4.8	54
2	Adriamycin	1,5,9	1.73-8	2.88	114	1.73	14
	Cinerubin A	1,5,9	1.73-8	4.8	103	4.8	29
3	Adriamycin	1,5,9	1.73-8	2.88	167	2.88	20
	Adriamycin	1-9	0.65-3	1.8	127(2/7)†	1.8	9
	Cinerubin A	1,5,9	1.73-8	4.8	90	4.8	37
	Cinerubin A	1-9	0.65-3	1.8	139	1.8	33
4	Adriamycin	0-4	1.08-3	—	—	1.8	18
	Daunorubicin	0-4	1.08-3	—	—	1.08	16
	Cinerubin A	0-4	1.08-3	—	—	1.8	34
5	Adriamycin	2,6,10	1.50-6	3	135	1.5	12
	Cinerubin A	2,6,10	2.50-10	5	102	2.5	26
6	Adriamycin	1,5,9	1.73-8	2.88	101	1.73	7
	Rubidazone	1,5,9	6.50-30	6.5	62	6.5	3
	Carminomycin	1,5,9	0.39-1.8	0.39	97	0.39	9
7	Adriamycin	1,5,9	1.73-8	2.88	158	2.88	21
	Carminomycin	1,5,9	0.32-1.5	0.32	74	0.32	11
8	Adriamycin	1,5,9	1.73-8	2.88	150(1/8)†	2.88	25
	Adriamycin-14-octanoate		2.88-13.33	4.8	209(2/8)†	4.8	21
9	Adriamycin	1,5,9	1.73-8	2.88	134	2.88	16
	Nogalamycin	1,5,9	0.65-3	1.08	41	1.08	0

*%ILS based on mean survival time (excluding survivors) at optimal dose.
†Numbers in parentheses = No. of survivors on Day 60/No. of animals treated.

those involving Lewis lung carcinoma, in which case drugs were administered iv.

RESULTS

A subline of P388 leukemia with acquired resistance to adriamycin exhibited essentially complete cross-resistance (maximum increase in lifespan [ILS] < 25%) to the anthracycline derivatives: daunorubicin, adriamycin-14-octanoate, rubidazone, carminomycin, and nogalamycin (table 1). In contrast, cinerubin A, at optimal dose levels, increased the lifespan of mice bearing the adriamycin-resistant tumor by 26%-54% in a number of experiments.

In view of the residual activity of cinerubin A against the adriamycin-resistant subline (P388/ADR) and activity approximately equivalent to adriamycin against the sensitive line of P388 leukemia (P388/S) (table 1), the two drugs were compared in a number of tumor systems. On a variety of treatment schedules, cinerubin A proved to be clearly inferior to adriamycin against ip L1210 leukemia, iv Lewis lung carcinoma, and ip B16 melanoma (table 2). In the latter two tumor systems, cinerubin A was only marginally effective whereas adriamycin was curative.

DISCUSSION

P388/ADR exhibited cross-resistance to a spectrum of anthracyclines. Cinerubin A was unique in that it had low but reproducible activity against P388/ADR. There are differences in the interaction with nucleic acids of cinerubin A and of other anthracyclines, such as daunorubicin and nogalamycin, which are reflected in differences in physicochemical properties of anthracycline-DNA complexes (2) as well as in differences in the relative inhibition of various DNA- and RNA-dependent

TABLE 2.—Comparison of adriamycin and cinerubin A against transplanted tumors

Drug	Treatment schedule	L1210 leukemia* (ip)			Lewis lung carcinoma† (iv)			B16 melanoma‡ (ip)		
		Dose range	Optimal dose	%ILS§	Dose range	Optimal dose	%ILS§	Dose range	Optimal dose	%ILS§
Adriamycin	Daily, Days 1-9	0.65-5	1.08	72	0.88-3.7	2.6	79 (2/8)	0.25-2	0.5	>172 (6/10)
	q4d, Days 1,5,9	1.04-8	4.8	45	1.92-8	5.6	124 (1/8)	0.25-4	2	>172 (5/10)
	Day 1 only	2.59-20	7.2	69	4.8-20	9.8	>200 (6/8)	—		—
Cinerubin A	Daily, Days 1-9	0.65-5	1.08	35	0.88-3.7	1.8	31 (1/8)	0.38-6	1.5	50
	q4d, Days 1,5,9	1.04-8	4.8	45	1.92-8	5.6	29	1.5-12	3	15
	Day 1 only	2.59-20	7.2	44	4.8-20	2.8	36 (1/8)	—		—

*Mean survival time of untreated controls = 8.9 days, 8 mice/group.
†Median survival time of untreated controls = 21.0 days.
‡Median survival time of untreated controls = 22.0 days.
§%ILS at optimal dose (No. of long-term, tumor-free survivors/total No. mice treated).

polymerases.[4] The lack of complete cross-resistance to cinerubin A of P388/ADR may be due to these differences in interaction with nucleic acid. However, the mechanism of resistance to adriamycin has not as yet been determined and could be due to a number of factors such as alteration in permeability of resistant tumor cells to the drug. The residual activity of cinerubin A could be due to differences in the uptake mechanism as compared to adriamycin.

The recent demonstration by Zbinden and Bachmann[5] of a similar degree of cardiotoxicity for adriamycin and cinerubin A in the rat is most significant. Regardless of the observed lack of complete cross-resistance with adriamycin, evidence of cardiotoxicity, taken in conjunction with the superior activity of adriamycin in a number of experimental tumor systems, argues against consideration of cinerubin A as a candidate for clinical trial. In addition, cardiotoxicity in two structures as divergent as those of adriamycin and cinerubin A suggests that major modifications of the adriamycin structure may be necessary to find an analog which is not cardiotoxic. Cinerubin A differs from adriamycin and daunorubicin in the sugar side chain, in the position and nature of functional groups on the aglycone, and at carbon atom 13, a major site of metabolism for both adriamycin and daunorubicin (9). Of the remaining structural similarities, the hydroxyl groups adjacent to the quinone carbonyls may account for cardiotoxicity antitumor activity as well). The involvement of these substituents in chelation of divalent cations (10) or clevage of the aglycone (11,12) has been suggested as a basis for cardiotoxicity.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8. Supplied by Farmitalia, Milan, Italy.

Adriamycin-14-octanoate: NSC-149584; adriamycin, 14-octanoate, hydrochloride. Supplied by Farmitalia, Milan, Italy.

Carminomycin: NSC-180024; 5,12-naphthacenedione, 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranoxyl)]-7,8,9.10-tetrahydro-1,6,8,11-tetrahydroxy-, (8*S-cis*). Supplied by the Institute of Antibiotics, Moscow, USSR.

Cinerubin A: NSC-18334. Supplied by Ciba-Geigy, Ltd, Basel, Switzerland.

Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6. Supplied by Farmitalia, Milan, Italy.

Nogalamycin: NSC-70845; CAS reg. No. 1404-15-5. Supplied by The Upjohn Company, Kalamazoo, Mich.

Rubidazone: NSC-164011; CAS reg. No. 36508-71-1; daunorubicin, benzoylhydrazone, monohydrochloride. Supplied by Stanford Research Institute, Menlo Park, Calif.

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Sequential Combination Chemotherapy for Advanced, Recurrent, Squamous Carcinoma of the Head and Neck[1,2]

Brief Reports and Preliminary Communications

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Squamous carcinoma of the head and neck is a problem commonly seen by the chemotherapist in regional cancer centers, usually after surgical and/or radiation therapy have been used to their fullest extent with ultimate failure to control the disease. A variety of single agents (including methotrexate, bleomycin, cyclophosphamide, adriamycin, and vinblastine) have been used with some striking antitumor effects as measured by response rate. However, although one third or more of patients will respond to single-drug therapy, the responses seen are usually partial and the response duration is short, with a median of 6-12 weeks in most reported series (1). Previous efforts with combination chemotherapy (2,3)[7] have resulted in response rates no better and response durations no longer than those reported for methotrexate alone (4), and in our immediately previous five-drug regimen, one third of the patients with cancer of the head and neck required hospitalization for drug-related toxicity.[7]

In the current study, sequential combined drugs were employed in an attempt to achieve three major goals: (*a*) delay the development of clinically evident drug resistance and thus prolong responses; (*b*) make use of the most effective single agent, methotrexate, which is difficult to use in concert with other stomatitis-producing agents like bleomycin and adriamycin; and (*c*) reduce morbidity and mortality related to treatment. It is important to emphasize that in this regimen, in order to attempt to delay drug resistance, the switch to the next part of the program was made at fixed predetermined intervals and did not require that advancing disease develop in the patient for the change to occur (although if early progression occurred, we did make the switch prematurely).

MATERIALS AND METHODS

The regimen employed is shown in table 1. A total of 28 patients with advanced, recurrent, squamous carcinoma of the head and neck who had received no prior chemotherapy were treated. In addition, six patients with squamous carcinoma of the esophagus, one with adenocystic carcinoma of the parotid, and one with a tumor of unknown primary site were treated. Eight patients with squamous carcinoma of the head and neck who had had prior methotrexate chemotherapy were treated with the regimen described, with the exception that the methotrexate cycle was deleted.

Of the 28 patients with squamous carcinoma of the head and neck who had had no prior chemotherapy, 22 (79%) had received prior radiotherapy, 23 (82%) had had prior surgical resection of the primary tumor, and 27 (97%) had received attempted definitive treatment with one or the other of these modalities. Six patients were treated at the University of Indiana; all other patients had been referred from the Head and Neck Section of the Department of Surgery at M.D. Anderson Hospital and Tumor Institute. The median age was 59 years, with a range of 39-73 years.

RESULTS

Of the 28 patients with head and neck squamous cancer who had received no prior chemotherapy, 12 (43%) had a > 50% regression of measurable tumor, and five (18%) of these patients had complete clinical disappearance of tumor. One of the 28 patients died within 2 weeks of the start of the study. Response analysis by subsite of the primary lesion

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[5]Deceased.

[6]The authors gratefully acknowledge the secretarial assistance of Ms. Terri Oshman.

[7]Richman SP, Livingston RB, Suen JY, et al. Chemotherapy versus chemoimmunotherapy of head and neck cancer: report of a randomized study. Submitted to Cancer Treat Rep, 1975.

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revealed that five of seven patients with primary lesions of the tongue responded (four with CRs), and a lower responsiveness in patients with the primary lesion in the larynx was suggested (no responses among four patients). Of the 12 responses, nine (75%) occurred during the initial treatment cycle with cyclophosphamide, vincristine, and bleomycin, two occurred during the methotrexate cycle after a period of no change and progression of disease respectively during the initial cycle, and one occurred during the adriamycin and methyl-CCNU cycle after disease progression with methotrexate.

Of the six patients with esophageal carcinoma, one had a PR during the first cycle. The patient with an adenocystic tumor responded to adriamycin and methyl-CCNU after 18 weeks of "stable disease" during the first two cycles. The patient with an unknown primary site had complete regression of a pulmonary mass during methotrexate treatment, having achieved a PR during the first cycle.

Three of the eight patients with cancer of the head and neck who had received prior chemotherapy and had had progression of disease achieved PRs: two during the first cycle and one during the adriamycin and methyl-CCNU cycle after having had progression of disease during the first cycle.

The toxicity of the first cycle (cyclophosphamide, vincristine, and bleomycin) was analyzed in 39 patients who received 56 courses at the intended (full) dose. The median nadir granulocyte count was 1500 cells/mm^3 with a fall to 500 cells/mm^3 or less in 11 of 56 courses (19%). Thrombocytopenia was observed only in two patients who received cyclophosphamide in a manner violating the protocol after exposure to adriamycin and methyl-CCNU. No cumulative effect on granulocyte counts was seen after the second dose of cyclophosphamide. The serious morbidity seen included four of 39 patients with leukopenia in whom a presumed infection developed which was treated with antibiotics; two of these patients had drug-related deaths with documented sepsis. These were the same two patients who had received a 1-g/m^2 dose of cyclophosphamide after prior treatment with a nitrosourea in violation of the protocol. One patient died from drug-related interstitial pulmonary fibrosis, probably secondary to bleomycin (total dose, 180 units). Most patients had nausea and vomiting produced by cyclophosphamide. No hemorrhagic cystitis was observed. Alopecia was almost universal.

The toxicity of the methotrexate cycle was analyzed in 21 patients, all of whom began at a dose level of 30 mg/m^2. In one half of the patients the doses could be escalated to 60 mg/m^2 before significant side effects occurred. Stomatitis was the most common dose-limiting effect and occurred in 13 patients (62%). Myelosuppression was observed in eight patients (38%), and chemical hepatitis without clinical sequelae occurred in three (15%). These side effects were seen at all dose levels and represent the result of intentionally escalating each

TABLE 1.—Doses and schedules for 6-drug regimen

Cycle*	Drugs	Doses and routes	Schedule
1 (1st 6 wks)	Cyclophosphamide	1 g/m^2 iv	Days 1, 22
	Vincristine	0.75–1.0 mg iv in the morning	Day 2, then weekly × 6
	Bleomycin	30 units iv 6 hrs after vincristine	Day 2, then weekly × 6
2 (next 12 wks)	Methotrexate	30 mg/m^2 im increased at weekly intervals by 10 mg/m^2 to dose producing mild toxicity	Weekly × 12
3 (next 6 wks)	Adriamycin	*a.* 60 mg/m^2 iv *b.* 45 mg/m^2 iv	*a.* Day 1 *b.* Day 22 (counts permitting)
	Methyl-CCNU	100 mg/m^2 orally	Day 1 only
4 (next 6 wks)	Methotrexate	At previously tolerated dose	Weekly × 6
5 (next 6 wks)	Adriamycin	Same as in cycle 3	Same as in cycle 3
	Methyl-CCNU	Same as in cycle 3	Same as in cycle 3

*Cycles 4 and 5 were alternated continuously ad libitum, with adriamycin stopped at 450 mg/m^2 to prevent cardiotoxicity.

patient's dose to biologic effect. Only one patient was hospitalized to receive antibiotics while having leukopenia caused by methotrexate, and one patient was taken off the drug because of unacceptable stomatitis. It should be noted that stomatitis often occurred without myelosuppression and vice versa, as has been observed by others.

The toxicity of the adriamycin-methyl-CCNU cycle was analyzed in nine patients who received 15 courses. The median nadir granulocyte count was 800 cells/mm^3 and occurred on median Day 15 (range, Days 13-41). Five of 15 courses (33%) were characterized by a fall to 500 or fewer granulocytes/mm^3. The median nadir platelet count was 120,000 platelets/mm^3 and occurred on median Day 25. Four of 15 courses (27%) were characterized by a fall to 50,000 or fewer platelets/mm^3. Three patients (33%) were hospitalized to receive antibiotics while having leukopenia during this cycle, and one patient's death was documented as being caused by sepsis.

This six-drug regimen resulted in hospitalization related to treatment for 14% of the patients versus 32% in our previous five-drug simultaneous regimen, BACON (1). As opposed to our previous experience, where all drug deaths occurred in patients after the prescribed regimen, two of the three drug deaths caused by the current regimen were related to inappropriate administration of full-dose cyclophosphamide after the patient had received the nitrosourea.

The median duration of response for this six-drug therapy was 25 weeks (approximately 6 months). The median survival time for all patients entered in the study was 24 weeks from the start of chemotherapy. Responders lived significantly longer than nonresponders (median 38 weeks vs 10.5 weeks, $P < 0.01$).

DISCUSSION

The response rate to this regimen is no higher than can be achieved with methotrexate alone. The median response duration of 25 weeks, however, is considerably longer than reported durations of response for methotrexate or other single agents in the treatment of head and neck cancer (5). Dowell et al (6) from the Central Oncology Group have reported response rates of 30%-44% in four- and five-drug combinations used in the treatment of head and neck cancer, but the mean response duration was only 9 weeks.

We believe that cyclophosphamide-vincristine-bleomycin, methotrexate, and adriamycin-methyl-CCNU represents an intermediate step forward in the treatment of squamous carcinoma of the head and neck, in that the response duration may be prolonged and toxicity, relative to our previous experience with simultaneous combinations, has been lessened. The appearance of CRs in 18% of the patients is particularly encouraging. The addition of nonspecific immunotherapy appears to make a further impact on response duration and survival.[8] We hope to increase the response rate and the quality of tumor regression, retaining the cyclic principle, by further manipulations of drug combination and sequence and by stimulation of host defense mechanisms.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

BACON = bleomycin, adriamycin, CCNU, vincristine (Oncovin), and mechlorethamine (nitrogen mustard)

Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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[8] See footnote 7.

Phase II Study of Combination Cytotoxic Chemotherapy in Advanced Breast Cancer: 2 Years' Followup[1]

Brief Reports and Preliminary Communications

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In the past 10 years progress has been made in the management of patients with advanced breast cancer using combination cytotoxic chemotherapy. This type of treatment is considered preferable to single-agent chemotherapy. Schedules based on Cooper's regimen (1) have been tested with the aim of lowering toxicity and increasing individual tolerance. The protocol we have used is a modification (2) of Cooper's regimen. Another purpose of this trial was to set up a maintenance regimen to be used after an objective remission was obtained. From July 1972 until July 1974 we carried out a phase II study in 32 patients with advanced breast cancer.

PATIENTS AND METHODS

Thirty-two consecutive evaluable women (age range 36-70 years; median age 51) were entered in the study. The primary treatment had been mastectomy in 18 patients, radiotherapy (cobalt 60) in eight, preoperative radiotherapy and surgical treatment in five, and cytotoxic chemotherapy in one. With regard to previous chemotherapy, seven patients had received single-agent chemotherapy and seven had received combination chemotherapy. Seventeen patients were premenopausal, four were $\leq$ 1 year postmenopausal, and 11 were > 1 year postmenopausal. Fourteen patients had had a disease-free interval for < 2 years, six for between 2 and 5 years, and three for > 5 years. The dominant lesions were visceral in 12 patients, osseous in nine, and soft tissue in 11.[4] The criteria used for objective evaluation of response have been described by Carter (3). Before each course of therapy white blood cell (wbc) and platelet counts were done.

The schedule used to induce remissions was as follows: 5-fluorouracil (5-FU) (500 mg), methotrexate (25 mg), and vincristine (1 mg) given iv weekly; cyclophosphamide (100 mg) given orally on Days 1-15 with a 2-week rest period; and prednisone (45 mg/day, initially) given orally with progressively diminishing dose.[5]

If myelotoxicity developed, the interval between the courses was increased; however, the increased interval was never > 2-3 weeks since this allowed enough time for the bone marrow to completely recover.

A maintenance regimen was started when either a CR or a PR had been obtained, but in the case of PR, the maintenance regimen was not started before 15-20 courses of therapy had been given. The maintenance regimen was 5-FU (750 mg), methotrexate (25 mg), and vincristine (1 mg) given iv on Days 1, 8, and 15, and cyclophosphamide (100 mg) given orally on Days 1-15. There were 3-week rest intervals between the courses.

RESULTS

In the 1st year of treatment, 22 of 32 patients (68.8%) had attained objective remissions: 17 PRs and five CRs. The median duration of remission was 6.4 months (range 4-12 months). At the end of the 2nd year of the trial, only eight patients were in remission (25%); the median duration was 8.5 months (range 4-22 months).[6] The number of cours-

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[3]The authors wish to thank Drs. S. Carter (Division of Cancer Treatment, National Cancer Institute) and R.D. Rubens (Imperial Cancer Research Fund, Breast Cancer Unit, Guy's Hospital, London) for helpful advice and suggestions.

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[4]Where multiple sites were involved, we have classified the patients by category of the worst prognosis. The order of increasing severity was soft tissue, osseous, and visceral metastases.

[5]5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,-3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

[6]By Nov 1975, the eight patients were still in remission. The range is now from 4 to 26 months.

es each patient received during the 2 years varied between eight and 57. At the end of the 1st year, of the 18 patients in whom the primary treatment had been mastectomy, remissions were attained in 13. At the end of the 2nd year, five of these responders were still alive and in remission. Of the eight patients treated primarily with radiotherapy, remission occurred in three by the end of the 1st year and in none at 2 years. Of the five patients treated with preoperative radiotherapy followed by surgical treatment, three were in remission at the end of the 1st year and two of the three were still in remission at the end of the 2nd year. Only one patient had received combination cytotoxic chemotherapy as primary treatment. This patient is still in remission. Our results showing the relationships between menopausal status, disease-free interval, and site of dominant lesion are summarized in table 1.

TOXICITY

The most frequent toxic reaction was peripheral neuropathy produced by vincristine and observed in 22 patients. This reaction never achieved a rating greater than grade I, according to the criteria of Holland et al (4), because, at that time, administration of the drug was withheld until complete recovery. The 3-week rest period in the maintenance schedule was sometimes sufficient to correct this.

The second most frequent manifestation of toxicity was alopecia, which was observed in 21 patients. By alternating the courses of cyclophosphamide, alopecia was less evident and there was faster recovery. Leukopenia of < 4000 cells/mm^3 occurred in two thirds of the patients but counts were never < 1000 cells/mm^3. A platelet count of $<100{,}000$ platelets/mm^3 never occurred. Gastrointestinal toxicity did not occur. Diabetes mellitus occurred in one patient.

DISCUSSION

One of the features of this trial was the heterogeneous nature of the group of patients treated. This selected group had, however, one important similarity in that all of the patients were no longer able to receive surgical, radiation, or hormonal therapy.

The results obtained during the 1st year of treatment (68.8% regressions) were similar to those recorded in the literature (2,5,6). We can therefore confirm that in the treatment of advanced breast cancer progress has been made with combination chemotherapy. The best results ob-

TABLE 1.—Relationship between menopausal status, disease-free interval, and site of dominant lesions

		No. of patients					
		1st Year (32 patients)			2nd Year (22 patients)		
		Nonresponders* (10 patients)			Nonresponders (14 patients)		
	No. of patients treated	Dead	Living	Responders† (22 patients)	Dead	Living	Responders (8 patients)
Premenopausal	17	0	4(23.5%)	13(76.5%)	5(29.4%)	3(17.7%)	5(29.4%)
Postmenopausal							
< 1 yr	4	0	2(50.0%)	2(50.0%)	1(25.0%)	0	1(25.0%)
> 1 yr	11	0	4(36.4%)	7†(63.6%)	3(27.2%)	0	2(18.1%)
Free interval							
0-2 yrs	14	0	2(14.2%)	12(85.8%)	3(21.4%)	3(21.4%)	6(42.9%)
2-5 yrs	6	0	0	6†(100.0%)	3(50.0%)	0	1(16.7%)
> 5 yrs	3	0	3(100.0%)	0	0	0	0
Predominant lesions							
Soft tissue	11	0	3(27.3%)	8(72.7%)	6(54.5%)	0	2(18.2%)
Osseous	9	0	3(33.4%)	6(66.6%)	0	2(22.2%)	4(44.4%)
Visceral							
1. Pulmonary	7	0	1(14.3%)	6†(85.7%)	1(16.6%)	1(16.6%)	2(33.3%)
2. Nonpulmonary	5	0	3(60.0%)	2(40.0%)	2(40.0%)	0	0

*10 patients were excluded from the protocol at the end of the 1st yr because of progression of disease.
†2 deaths occurred away from our oncology center.

tained have been in premenopausal women and women who are < 1 year postmenopausal (7). A better response rate in our regimen was observed in women with a shorter disease-free interval, as previously reported by others (6,7). With respect to the dominant lesion, virtually identical response rates were seen in patients with soft tissue, pulmonary, and osseous lesions (in this small series); we have seen rapid regressions of pulmonary metastases in six of seven patients by the end of the 1st year of the trial.

If we speculate that systemic chemotherapy acts on the pulmonary parenchyma as an intra-arterial perfusion, it can be postulated that this is the mechanism responsible for the high remission rate in patients with pulmonary metastases. The best results were obtained in patients who had had no previous cytotoxic chemotherapy and in patients in whom the primary treatment had been mastectomy; poor results were obtained in patients who had previously received combination chemotherapy or radiotherapy (alone or followed by surgical treatment).

Once cytotoxic combination chemotherapy has been initiated, we believe it should not be interrupted. The maintenance schedule, with 3-week rest periods, allows treatment of patients for long periods, thus avoiding or minimizing severe myelosuppression and/or neurotoxicity. In trying to increase this interval from 3 to 4 weeks, we observed rapid progression of the disease. We recommend this protocol for routine clinical use in view of the increase in individual tolerance, diminution of toxic effects, improvement of the response rate (8), and significant palliation in patients with poor prognoses.

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Treatment of Kaposi's Sarcoma With ICRF-159 (NSC-129943)[1,2,3]

C. L. M. Olweny,[4,*] J.P. Masaba,[5] W. Sikyewunda,[5] and T. Toya[4]

ICRF-159 was developed at the Imperial Cancer Research Fund facilities in London, England. It is a derivative of ethylenediamine tetraacetic acid and has antitumor activity in a variety of tumors including Sarcoma 180, L1210 leukemia, Carcinoma 755, and Walker carcinoma 256 (1). Mice bearing Lewis lung tumor treated with ICRF-159 at doses which had little effect on the primary tumor did not develop pulmonary metastasis (2). This might be due to the normalization of blood vessel development at the invading margin of the primary tumor (3). Clinical trials have demonstrated therapeutic activity against rapidly advancing leukemia, including cases which had resisted treatment with conventional anticancer drugs (4). Kaposi's sarcoma is a very vascular tumor and we felt that the angiometamorphic effect of ICRF-159 would greatly influence its growth. The results of treating 18 patients with Kaposi's sarcoma using ICRF-159 are presented.

MATERIALS AND METHODS

Patients with histologically proven Kaposi's sarcoma were entered into this study. All patients had either clinically or radiologically measurable lesions. Twelve of the 18 patients had relapsed after previous drug treatment and six patients had developed resistance to conventional therapy. The clinical and histologic classifications used in this study have been previously described (5). A pretreatment evaluation included a history, physical examination, centimeter measurements of lesions, complete blood cell count, urea, uric acid, and liver function tests. Roentgenographic examination of the chest and other possible areas of bone involvement, clinical photographs, urinalysis, and stool examinations were performed routinely. After completion of these studies patients received 1 g/m^2/day of ICRF-159 orally for 3 days, and each day's dose was equally divided into eight hourly doses; this constituted a course and was repeated 2 weeks later. We define complete response as the complete disappearance of all observable and measurable tumor, partial response as the reduction of tumor volume by at least 50%, and no response as a lesser response or disease progression.

RESULTS

The clinical characteristics of the 18 patients studied are summarized in table 1. The median age was 38.5 years (range, 10-67 years).

Only one patient (No. 2) exhibited complete tumor regression after four courses of treatment and has been in remission for 5+ months. He had florid monomorphic type of disease. Ten patients (55.5%) had partial response and seven patients (39%) had no response at all. On the average, it took six courses (range, three to nine) to observe any drug antitumor effect. The toxic effects observed were myelosuppression, gastrointestinal upset, and alopecia. Leukopenia was more common than either thrombocytopenia or anemia, and the median white blood cell count nadir was 2400 cells/mm^3 (range, 2000-4800 cells/mm^3).

DISCUSSION

ICRF-159 is an antitumor agent that acts by inhibiting mitosis during the G_2-M phase of the cell cycle (6,7), a phase which is considered unaffected by most conventional cytotoxic drugs. This would make it ideal for the treatment of patients who are resistant to other drugs and for possible combination chemotherapy.

In this study, a 60% overall response rate was observed. However, Kaposi's sarcoma is capable of running a protracted course and may regress spontaneously. This is particularly true of the nodular mixed form of the disease. It responds to oral alkylating agents and may undergo spontaneous regression (5). The florid, lymphadenopathic, or infiltrative lesions, especially those with monomorphic or anaplastic histology, are malignant and unlikely to regress independently. The patient (No. 2)

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[3]ICRF-159: CAS reg. No. 21416-87-5; 2,6-piperazinedione, 4,4'-propylenedi-, (±)-. Supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI.

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TABLE 1.—Characteristics of 18 Kaposi's sarcoma patients treated with ICRF-159

Patient No.	Age (yrs)/ sex	Disease type		Previous treatment			ICRF-159 treatment			
		Clinical	Histologic	Drugs*	No. of courses	Type of response and duration (mos)	No. of courses	Total dose (g) received	Type of response	Toxic effects
1	40/M	Florid	Monocellular	a. BCNU+Act-D	7	CR (7)	4	13.5	PR	Myelosuppression
				b. Act-D+VCR+DTIC	4	CR (4)				
2	65/M	Florid	Monocellular	a. Act-D	3	CR (14)	6	32.4	CR†	Anorexia, headache, myelosuppression
				b. Act-D+DTIC	6	CR (15)				
3	31/M	Nodular	Mixed	a. BCNU+Act-D	5	NR	6	27	PR	None
				b. DTIC	2	NR				
				c. Bleomycin	10	PR				
				d. DTIC+Act-D+VCR	8	CR (4)				
4	65/M	Florid	Mixed	Act-D+DTIC	8	CR (17)	7	31.5	PR	Stomatitis, diarrhea, vomiting, myelosuppression
5	37/M	Florid	Monocellular	a. Act-D+BCNU	6	PR	6	27.0	NR	Multiple aches and pains
				b. Act-D+BCNU	2	Defaulted				
				c. Act-D+VCR+DTIC	5	PR and defaulted				
6	45/M	Florid	Mixed	a. DTIC+Act-D+VCR	7	PR	7	28.7	PR	Vomiting, anorexia
				b. ADM	2	PR				
				c. Trenimon		NR				
7	18/M	Nodular	Mixed	a. Act-D	6	NR	8	28.8	PR	Myelosuppression
				b. Bleomycin	10	PR				
8	38/M	Nodular	Mixed	DTIC+Act-D+VCR	8	CR (9)	7	34.7	PR	None
9	67/M	Nodular	Mixed	Act-D+VCR	7	CR (11)	9	40.5	PR	Vomiting
10	39/F	Nodular	Mixed	a. Act-D+VCR	4	CR (8)	4	33.99	PR	Vomiting, diarrhea
				b. Act-D+VCR	3	CR (9)				
				c. Act-D+VCR	4	CR (5)				
11	30/M	Nodular	Mixed	Act-D+VCR	8	CR (14)	7	44.1	PR	None
12	36/M	Florid	Monocellular	Act-D+VCR	6	PR	4	18	NR	Alopecia
13	30/M	Nodular	Mixed	Act-D+VCR	8	CR (12)	6	25.7	PR	Alopecia
14	11/F	Lymphadenopathic	Mixed	a. Act-D+VCR	12	PR	6	18.9	NR	Myelosuppression, anorexia
				b. BCNU		NR				
				c. Act-D+VCR	3	PR				
				d. Radiotherapy		CR				
				e. Act-D+VCR+DTIC	6					
15	10/F	Lymphadenopathic	Mixed	Act-D	2	NR			NR	None

16	46/M	Infiltrative	Mixed	a. Act-D+VCR+DTIC	6	CR (4)	11	23	NR	Abdominal pain, diarrhea
				b. ADM	4	PR			Died	
				c. Act-D+VCR+DTIC+ADM	1	PR				
17	58/M	Nodular	Monocellular	a. Act-D+VCR	4	PR	7	22.4	NR	Abdominal pain
				b. Act-D+VCR+DTIC	2	NR				
18	45/M	Nodular	Mixed	a. Act-D+VCR	4	CR (4)	2	9	NR	Alopecia
				b. Act-D+VCR+BCNU		PR				
				c. Bleomycin	10	PR				
				d. Act-D+VCR+DTIC	1	PR				
				e. Act-D+VCR+DTIC	3					

*Act-D = actinomycin D; VCR = vincristine; ADM = adriamycin; Trenimon = 2,3,5-tris(1-aziridinyl)-*p*-benzoquinone.
†Complete tumor regression after 4 courses; patient has been in remission for 20+ weeks.

who achieved complete tumor regression had relapsed after previous therapy with actinomycin D alone and in combination with DTIC. This patient had florid monomorphic disease, a type with a paucity of vessels. This suggests that the action of ICRF-159 may not be directly related to its presumed angiometamorphic effect.

The toxic effects observed were mainly leukopenia, gastrointestinal upset, and alopecia. The blood cell counts usually returned to normal within 2 weeks. No drug-related deaths were observed. At the doses used in this study the drug was tolerable, easy to administer, and exhibited a fairly high therapeutic index in Kaposi's sarcoma.

ADDENDUM

Since this manuscript was accepted for publication one other patient (No. 6) has achieved complete tumor regression after 11 courses of ICRF-159.

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Adriamycin (NSC-123127) Versus 5-Fluorouracil (NSC-19893) and Cyclophosphamide (NSC-26271) in the Treatment of Metastatic Prostate Cancer[1,2]

Brief Reports and Preliminary Communications

Robert T. Eagan,* Richard G. Hahn, and Robert P. Myers[3]

The major impediment to the quick and accurate assessment of the value of chemotherapeutic agents in metastatic prostatic cancer is the fact that the predominant disease seen by most chemotherapists is blastic skeletal metastases. Using this as the only objective parameter of disease makes the analysis of the efficacy of treatment quite difficult. This study, which represents the completion of a previously published preliminary report (1), was designed with two major purposes in mind: (*a*) to establish an ancillary scoring system to be used in assessing drug response, and (*b*) to evaluate two different chemotherapeutic programs employing both the new system and conventional methods.

MATERIALS AND METHODS

Twenty-six men with metastatic adenocarcinoma of the prostate were entered in the study. All had failed both estrogen therapy and orchiectomy (except one who refused orchiectomy). None were receiving estrogen at the time of the study. The median age was 64 years with a range of 51-78 years. At the start of chemotherapy, five patients had liver metastases, two had lung metastases, and two had distant nodal metastases (measurable disease). During the course of the study, one patient developed lung metastases and another developed liver metastases. No liver or lung biopsies were done to confirm the clinical, roentgenographic, and/or scan diagnoses, nor were autopsies done for this group of patients.

The eligibility criteria for entry in the study and the patient stratification for age, site of predominant disease, and prior irradiation have been detailed elsewhere (1). After stratification, patients were randomly allocated to receive either adriamycin (60 mg/m^2 given as a single iv dose every 3 weeks × 2 and every 4 weeks thereafter) or the combination of 5-fluorouracil (5-FU) (300 mg/m^2) and cyclophosphamide (150 mg/m^2) administered iv daily for 5 consecutive days and repeated every 5 weeks from the first day of treatment. Three of 26 patients with "extensive" prior irradiation were begun at approximately two thirds of the above dosages. Dosages were adjusted to keep the white blood cell (wbc) nadir > 2000 cells/mm^3 and the platelet nadir > 75,000 platelets/mm^3. After disease progression on initial therapy, the patient was crossed over to the opposite treatment if protocol eligibility criteria were still met.

Evaluation of response to therapy was divided into two parts: actual tumor measurement (excluding prostate size) for patients with measurable disease and the assessment of ancillary factors (table 1) for patients with both measurable and skeletal metastases only. If only ancillary factors could be evaluated, a score of 9 or less was considered a response, 10-14 indicated stable disease, and

TABLE 1.—Ancillary factors analyzed and scores assigned for changes*

Ancillary factors	Changes and scores
Pain	None or normal = 0
Weight (≥10%)	Better = 1
Hemoglobin (≥10%)	Same = 2
Performance score (1 level)	Worse = 4
Alkaline phosphatase (≥25%)	
Acid phosphatase (≥25%)	

*9 or less = response; 10-14 = stable disease; 15 or more = progression.

[1]Received Aug 26, 1975; revised Nov 3, 1975; accepted Nov 19, 1975.

[2]Adriamycin: CAS reg. No. 23214-92-8.

5-Fluorouracil: CAS reg. No. 51-21-8.

Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Drugs were supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute.

[3]Division of Medical Oncology (Drs. Eagan and Hahn) and Department of Urology (Dr. Myers), Mayo Clinic, Rochester, Minn.

**Reprint requests to*: Robert T. Eagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, Minn 55901.

15 or greater indicated disease progression. Development of any new lesions, regardless of ancillary score, was considered progression. The details of other criteria for response were given in our preliminary report (1).

RESULTS

The two treatment groups were comparable with respect to age, Eastern Cooperative Oncology Group (ECOG) performance score (PS), and site of predominant disease. Nineteen patients were treated with adriamycin (14 as initial treatment and five on crossover) and 18 were treated with the combination of 5-FU and cyclophosphamide (12 as initial treatment and six on crossover).

Five of 19 patients (26%) responded to therapy with adriamycin, three of 14 on initial treatment and two of five on crossover. Two of 18 patients (11%) responded to the combination of 5-FU and cyclophosphamide, two of 12 on initial treatment and none of six on crossover. Responses were noted in lung, liver, nodal, and skeletal metastases. In all, seven of the 26 patients (26%) responded to one or the other of the two treatments.

Using the ancillary scoring system, all seven patients considered to have responded by more conventional criteria had ancillary scores of 9 or less. All but two of the patients who progressed had ancillary scores of 15 or greater. Of the two who did not, one developed new lytic bone metastases (score of 10) and one developed urethral obstruction by tumor (score of 13).

Among the seven responders, improvement of PS, stability or increase in weight, and decrease in bone pain were the most consistent changes in the ancillary factors occurring in all seven patients. The alkaline phosphatase level decreased or remained normal in six responders and increased in one of the seven. Acid phosphatase levels (both total and percent tartrate inhibited) decreased or remained normal in three responders and remained elevated but stable in four. All patients considered, alkaline phosphatase levels decreased in nine (six responders, one with stable disease, and two with disease progression), and acid phosphatase levels decreased in eight (three responders, one with stable disease, and four with disease progression). Both enzymes decreased in three patients (two responders and one with disease progression).

At present, 20 of the 26 patients are dead. The median survival for all 26 patients was 177 days. The seven responders had a median survival of 273 days (range, 95-411+ days) while the 19 patients who progressed had a median survival of 143 days (range, 21-375+ days). While a trend is apparent, the difference is not statistically significant at $P < 0.05$. There was also a suggestion that patients with visceral and/or nodal metastases in addition to skeletal metastases had a poorer median survival (140 vs 273 days), but this difference was also not statistically significant. There were no differences in response rate based on age. However, patients with better PSs seemed to respond better (three of nine with PS 1, three of six with PS 2, and one of 11 with PS 3) and live longer (300 vs 211 vs 127 days respectively).

TOXICITY

Eighteen of the 19 patients treated with adriamycin developed leukopenia with a median wbc nadir of 3000 cells/mm^3 on Day 13 with 6 days required for recovery. Four of 19 patients had mild thrombocytopenia. Fifty percent of the patients treated with 5-FU and cyclophosphamide developed leukopenia with a median wbc nadir of 2700 cells/mm^3 on Day 16 with 6 days required for recovery. Mild thrombocytopenia was noted in three. This toxicity is less, in our experience, than that seen in a group of previously untreated women with metastatic breast cancer using comparable drug dosages (2). Mild to moderate nausea and vomiting occurred in about one third of the patients on both regimens. Adriamycin produced alopecia in 100% of the patients versus about 50% for 5-FU and cyclophosphamide. No adriamycin cardiac toxicity was noted.

DISCUSSION

Because of the scarcity of published reports of randomized, prospective chemotherapy trials in advanced prostatic cancer, it is somewhat difficult to put the results of this study in perspective. The study was begun soon after the randomized trials conducted by the National Prostatic Cancer Project (NPCP) and the ECOG, and the choices of agents were made so that the other two studies would be complemented and would serve as extra-institutional "controls." The NPCP study randomized patients into three treatment groups: cyclophosphamide versus 5-FU versus "standard management." Preliminary data from the NPCP study (3) revealed 21%, 11%, and 0 response rates respectively for the first 66 patients. The ECOG study consisted of a randomization between adriamycin and 5-FU and some early results have been published (4). At the time of that report, one of eight patients had responded to adriamycin and

five of 14 patients had responded to 5-FU (used in the same dose and schedule as in the NPCP study). It can be seen that our reported 26% response rate for adriamycin seems reasonable. Our data for the combination of 5-FU and cyclophosphamide indicate that there is little gain from the combination of the two agents as compared to their use singly. No survival data have been reported from either the NPCP or ECOG studies.

The ancillary scoring system seems worthy of further examination and will be evaluated again in our next study. We will also try to study a mixture of patients with metastases of bone only and patients with bone plus visceral and/or distant nodal metastases which would reflect the percentage obtained in reported autopsy series (5).

REFERENCES

1. Eagan RT, Utz DC, Myers RP, et al. Comparison of adriamycin (NSC-123127) and the combination of 5-fluorouracil (NSC-19893) and cyclophosphamide (NSC-26271) in advanced prostatic cancer: a preliminary report. Cancer Chemother Rep 59:203-207, 1975.
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ANNOUNCEMENTS

Symposium on Recent Advances in Cancer Treatment Research

Baltimore, Maryland, March 25-27, 1976

This symposium will be Codirected by Dr. Theodore Woodward, Professor and Chairman, Department of Medicine, University of Maryland, and Dr. Peter Wiernik, Chief, Clinical Branch, Baltimore Cancer Research Center. A broad course focus will cover such topics as lung tumors, soft tissue sarcomas, Hodgkin's disease, and many other areas with internationally renowned speakers. This course is being tailored for the general physician as well as practicing or research-oriented oncologists. Since 1976 is the Bicentennial year, this as well as many of our other programs will also include a special planned activity (eg, tour, special teaching session, etc).

For further information please contact: Mitchell Perlin, Course/Media Coordinator, Program of Continuing Education, School of Medicine, University of Maryland, Baltimore, Md 21201.

Hematology/Oncology Fellowship

Hematology/Oncology fellowship applications are now being accepted for a 2-year fellowship training program at the Veterans' Administration Hospital in Long Beach, California.

This program offers opportunities in clinical or laboratory research. The hospital is a member of the Western Cancer Study Group and contributes to the National Institutes of Health's cooperative cancer chemotherapy programs. The section has 20 beds and active outpatient clinics. Present activities include research in cell cycle kinetics in myeloma and DNA metabolism. One or 2 years of medical residency is a prerequisite.

Individuals interested in a fellowship beginning July 1, 1976, are invited to submit a letter and curriculum vitae to: Dr. J. Brook, Chief, Hematology/Oncology Section, VA Hospital, 5901 E 7th St, Long Beach, Calif 90801.

Training Program in Medical Oncology

A 2-year training program in Medical Oncology is being offered at Tufts University School of Medicine and affiliated hospitals. Training is shared by the Medical Oncology units of the New England Medical Center, Lemuel Shattuck, and Pondville Hospitals. Elective rotations to Therapeutic Radiology, Surgical Oncology, Pathology, and Hematology are available. Fellowships require an internship and 1 or 2 years of previous residency training. The program is sponsored by the National Cancer Institute and carries a stipend of approximately $13,000 per year.

Further information may be obtained from: Larry Nathanson, MD, Chief, Medical Oncology Service, New England Medical Center Hospital, 171 Harrison Ave, Boston, Mass 02111.

Eighth World Congress of Gynecology and Obstetrics

Mexico City, October 17-22, 1976

The International Federation of Gynecology and Obstetrics announces the Eighth World Congress of Gynecology and Obstetrics to be held October 17-22, 1976, in Mexico City.

Only active members of national federations, societies, or colleges affiliated with the International Federation of Gynecology and Obstetrics may actively participate in this Congress.

There will be five plenary sessions, seminars on current topics of gynecology and obstetrics, correlated seminars, and three special invited lectures. Within this scientific program there will be free communications sessions, films, and precongress ccurses as well as scientific, technical, commercial, and artistic exhibits. In addition there will be a social program designed to enable attendants to become better acquainted.

The official languages of the Congress will be Spanish, English, and French with simultaneous translation into Spanish, English, French, and German for special lectures and plenary sessions.

Application forms to participate and to enroll in the Congress and further detailed information may be obtained from Carlos MacGregor, MD, General Secretariat, Avenida Baja California 317, Mexico 11, D.F., Mexico.

Medical Oncology Fellowship

Applications are now being accepted for 2-year fellowships in medical oncology which will begin July 1, 1976, at the University of Southern California School of Medicine. The service comprises clinics with over 500 monthly visits and 59 beds. Fellows have responsibility for patient management and house staff teaching. Standard and investigational chemotherapy and immunotherapy will be carried out under the supervi-

sion of Dr. Joseph R. Bateman who is Chairman of the Western Cancer Study Group and Professor of Medicine. Support facilities include laminar air-flow isolator units, a continuous-flow cell separator, and on-site cytogenetics and immunology laboratories. Rotations include student ward, consultation service, and electives (eg, hematology, hematopathology, tumor surgery, gynecologic oncology, radiation therapy, or laboratory research). Applicants should have completed 2 years of medical residency and be eligible for a California license. The current salary is $19,284 for the first year.

Address inquiries to: Dr. David Stolinsky, 2826 So. Hope St. Los Angeles, Calif 90007.

First International Symposium on HLA and Disease

Palais des Congres, Paris, France, June 23-25, 1976

This symposium will be concerned with the association of HLA antigens with numerous diseases. The clinical implications of these recent discoveries on nosology, diagnosis, prognosis, and even prevention will be discussed by clinicians and biologists.

There will be several workshops (eg, rheumatology, dermatology, gastroenterology, allergy, immunopathology, endocrinology) which will cover the different facets of this new approach to epidemiology and hereditary diseases. The possible mechanisms and biologic significance of this association will be considered by geneticists, virologists, and immunologists.

The Proceedings of this Symposium will be published by Munksgaard (Copenhagen) in a book covering the fundamental as well as the clinical aspects of these associations. *For further information, please contact:* CONGRES SERVICES I, Rue Jules Lefebvre, 75009 Paris, France.

Fellowship in Medical Oncology

Applications are being accepted from candidates who are interested in a Joint Collaborative Training Program in Medical Oncology involving the major teaching hospitals of Brown University's Program in Medicine beginning July 1976 and July 1977. This program offers clinical and research activities in clinical pharmacology and cancer chemotherapy, biochemical pharmacology, drug development, and tumor immunology. In addition, this program participates in national chemotherapy cooperative groups to aid in the treatment of patients with solid tumors and hematologic malignancies seen at many of the hospitals in the Providence, Rhode Island area. The prerequisites include 2 years of approved medical residency and American Board of Internal Medicine eligibility. Candidates with academic interests are encouraged to apply.

Send letter and curriculum vitae to: Paul Calabresi, MD, Professor and Chairman, Department of Medicine, or F. J. Cummings, MD, Head, Division of Medical Oncology, Department of Medicine, Roger Williams General Hospital, 825 Chalkstone Ave, Providence, RI 02908.

Postgraduate Course on Mammary Cancer

Milan, Italy, June 7-11, 1976

The National Cancer Institute of Milan, and the Milan section of the Italian League Against Cancer, will organize a Postgraduate Course on Mammary Cancer. The chairmen are U. Veronesi and A. Perussia and the Secretary is M. De Lena.

This course will include lectures on Epidemiology, Etiology, Pathology, Diagnostics, Clinical Classification, Treatment (surgical, radiologic, medical, combined, antalgic) and Sociologic Problems of mammary cancer. A large group of Italian experts will give their cooperation with foreign lectures and simultaneous translations will be provided.

Four panel discussions will be held on: "Early diagnosis of mammary cancer," "The operable mammary cancer: which surgery?", "Present and future endocrine therapy," and "Combined therapy: recent therapeutic approaches in mammary cancer." In addition, there will be closed circuit television presentations of surgical operations.

The registration fee is 80,000 lire (covering the course, the proceedings, lunch, etc). Because the number of participants is limited, please send your registration as soon as possible to: Miss Lucia Manfredi, Ufficio Attivita Didattiche, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian, 1, 20133 Milano, Italy (telephone No. 2363764).

First National Training Conference for Physicians on Psychosocial Care of the Dying Patient—Doctor-Patient Relationships in Terminal Illness

San Francisco, California, April 29-May 1, 1976

This program is presented by the Cancer Research Institute and Extended Programs in Medical Education, University of California School of Medicine, San Francisco, California. It is acceptable for 18 hours of Category I credit towards the certificate in Continuing Education for the California Medical Association and the American Medical Association.

The objectives of this course are to: (*a*) identify basic issues of psychosocial care; (*b*) identify the sequence of events encountered by physician and patient from diagnosis through death; and (*c*) apply an effective model dealing with the psychological issues surrounding death to clinical practice. The topics will be:

Psychosocial Aspects of the Dying Process;
Psychological Needs: Recognition and Action by the Physician;
Counselling the Patient's Family;
Medical Support: Patient Coping with Pain, Anger, Depression, Fear, and Treatment Side-Effects;
Team Efforts for Total Care: Doctor, Nurse, Family, Psychologist, Psychiatrist, Social Worker, Clergy, and Volunteer;
Anticipatory Grief and Bereavement;
Frequent and Extended Contact with the Dying: Impact on the Physician;
The Use of Psychological or Psychiatric Consultation and Trained Volunteers in Terminal Care;
Communicating with the Seriously Ill and Terminal Patient: When and How Should the Patient be Informed Regarding Diagnosis, Prognosis, Treatment, etc.

The fee for this conference will be $150.00. Students, interns, and residents (non-University of California) will be charged only half-fee with a letter of verification. Registration in advance is advised. In addition, there will be a $10.00 fee for refunds before the course begins and no refunds after the course begins. Publication of the proceedings will be mailed to all paid registrants after the course (included in fee).

To apply for registration or to receive more detailed information on the course write to: Extended Programs in Medical Education, Room C-135G, University of California, San Francisco, California 94143, or telephone 415-666-2483 (registration information) and 415-666-4251 (program information).

Applied Clinical Immunology—Methods, Applications, Interpretations

Houston, Texas, May 20-22, 1976

The University of Texas Health Science Center at Houston's Division of Continuing Education, in cooperation with Baylor College of Medicine and The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, will present a course on "Applied Clinical Immunology— Methods, Applications, Interpretations" to be held on May 20 and 21, 1976, with Laboratory Workshops on Saturday, May 22, 1976, at the Texas Medical Center in Houston, Texas.

This 2 ½-day course is structured to present to the practicing physician and the laboratory scientist current methods and approaches used in clinical medicine. Optional laboratory workshops will be given. The principles of immunology on which laboratory tests and immune profiling are based will be reviewed. The rationale, interpretation, and details of commonly employed immune techniques (or those tests likely to be adopted in the near future) will be presented. The course will include discussion of radio-immunoassays, cell-mediated tests, antibody determinations, and certain methods for assessing nonspecific immune function. There will also be visiting lecturers.

For further information contact: The Office of the Director, The University of Texas Health Science Center, Division of Continuing Education, PO Box 20367, Houston, Tex 77025 (telephone, 713/792-4671).

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